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Oxidative Chemical Transformations of Sesquiterpene Lactones.

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Oxidative Chemical Transformations of Sesquiterpene Lactones

A Dissertation

Submitted to the Graduate Faculty of the
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in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Chemistry

by

Howard Gordon Pentes
B.S., Louisiana State University, 1985

May 1991

To my dad, Gerson,

to my mom, Sonia,

and to my brothers,

Ronald and Steven

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I would like to express my gratitude and sincerest appreciation to Dr. Nikolaus H. Fischer, my major professor, without whose guidance this work would not have been possible. I thank him for sharing his unending supply of knowledge of natural products chemistry with me. His enthusiasm for and dedication to the pursuit of knowledge will inspire me for many years to come. I thank him for being my teacher, my mentor, and my friend. I would also like to thank Dr. Frank Cartledge, Dr. Dewey Carpenter, Dr. Joe Foley, and Dr. Steve Watkins for serving as my committee members.

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I would like to thank Dr. Frank Fronczek for solving all of the X-ray crystal structures included in this dissertation. I greatfully acknowledge the assistance of Raphael Cueto for the acquisition of some of the infrared data and Marco Gomez for the acquisition of most of the mass spectral data included in this dissertation.

I wish to thank Mrs. Helga Fischer and the past and present members of Dr. Fischer's research group for their help and friendship.

To all of my colleages, especially Marios Menelaou, Marco Gomez, Steve Arnold, and Drew Poche, thank you for giving me much needed friendship, advice, and encouragement.

I dedicate this dissertation to my parents, Gerson and Sonia, and to my two brothers, Ronald and Steven. My parents and my brothers have always encouraged

me to strive for excellence and they have always believed in my abilities. I thank them for their everlasting love and support.

I also dedicate this dissertation to the memory of three very special people: Harris Pentes, Libby Raphael, and Goldie Rodman. All three stressed the importance of education to me and were instrumental and generous in their support of my academic goals.

I would like to thank all of the members of my family, especially Hannah Bienn, Sara Friedlander, and Sarah Pentes, who each, in a small but significant way, contributed to the successful conclusion of this work.

And lastly, I thank Marc Hirsch, for his insights, his support, and his friendship.

Foreward

This dissertation is divided into five chapters. Chapter 1 is an introduction to sesquiterpene lactones, their biological activities, and the potential uses of their oxidatively modified derivatives.

In Chapter 2, the isolation of four naturally occurring sesquiterpene lactones which are used in subsequent oxidative transformations is described. In Part A, the procedure for the isolation of costunolide and dehydrocostuslactone from Costus Resinoid (*Saussurea lappa*) is described and in Part B the procedure for the isolation of dihydroparthenolide and psilostachyin A from *Ambrosia artemisiifolia* is described.

Chapter 3 is divided into two parts. In Part A, enolate oxidations of sesquiterpene lactones are carried out with oxygen. In Part B, (camphorylsulfonyl)oxaziridine is used instead of oxygen.

Chapter 4 is divided into four parts. Parts A and B describe the synthesis of two naturally occurring sesquiterpene lactones (11β,15-dihydroxysaussurea lactone and 15-hydroxy-dihydrocostunolide) from costunolide. In Part C, the synthesis and X-ray crystal structure of a hydroperoxy-sesquiterpene lactone is described. In Part D, biomimetic conversions of a germacrolide to a heliangolide are attempted.

Chapter 5 is divided into five parts (A-E) and describes miscellaneous modifications of sesquiiterpene lactones.

Chapters 2-5 are divided into parts which are written as individual publications. The references for each part of a chapter appear at the end of that part. Compounds, tables, figures, and schemes are all labelled sequentially within a chapter. The references are numbered sequentially after each part of a chapter.

Parts A and B of Chapter 3 and Parts A-D of Chapter 4 will be submitted to the

Journal of Organic Chemistry for publication. Chapters 2 and 5 are written in the same style so as to be consistent throughout this dissertation.

The ^{1}H and ^{13}C NMR spectra for most of the compounds described within each part of a chapter are recorded just preceeding the references for that part.

The data for compounds common to more than one chapter are not reported in subsequent chapters, however, notification of where this data is found in this dissertation is usually described.

Since Dr. Francisco Macias showed me the methodology he developed for enolate oxidations with oxygen, his name appears as a co-author of Chapter 3, Part A.

Since Dr. Frank Fronczek solved all of the X-ray crystal structures included in this dissertation, his name appears as a co-author of Chapter 3, Part A and Chapter 4, Part C.

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Abstract

Oxidative chemical transformations of sesquiterpene lactones have been carried out to determine the effect these changes have on the biological activities of these derivatives.

Vacuum liquid chromatography was used extensively to isolate the sesquiterpene lactones used for these synthetic transformations. Costunolide and dehydrocostuslactone were isolated from Costus Resinoid (*Saussurea lappa*) and dihydroparthenolide was isolated from *Ambrosia artemisiifolia.*

The enolate oxidations of sesquiterpene lactones with oxygen and (camphorylsulfonyl)oxaziridine have been investigated. Enolate oxidations of sesquiterpene lactones with oxygen generate low yields of both 11α- and 11β-hydroxylactones. The yields of enolate oxidations with (camphorylsulfonyl)oxaziridine are much improved (over oxygen) and the reaction is stereospecific. This methodology was used to prepare a series of 11-hydroxysesquiterpene lactones of various skeletal types.

Two naturally occurring sesquiterpene lactones (11β,15-dihydroxysaussurea lactone and 15-hydroxydihydrocostunolide) were synthesized from costunolide. These transformations involved the use of enolate oxidations developed previously, allylic oxidations with selenium dioxide, and Cope rearrangements of 1,5-dienes.

A hydroperoxy-sesquiterpene lactone (peroxydihydroparthenolide) was synthesized via an ene reaction between an alkene and singlet oxygen.

The first biomimetic conversion of a germacrolide to a heliangolide was achieved using tert-butyl hydroperoxide and selenium dioxide supported on silica

gel. Other attempted allylic oxidations of a 1,10-epoxygermacrolide with selenium dioxide resulted in isolation of only transannular cyclization products or oxidatively modified cyclization products.

Chapter 1. Introduction

For a long time, scientists have been interested in studying secondary metabolites, which are a class of natural compounds generally found only in plants and micro-organisms. Secondary metabolites are not intimately involved in essential life processes, like primary metabolites (e.g. amino acids) are, however they do seem to be important to the organism that produces them. Scientists have been curious about the structures of these compounds isolated from natural sources, their origins, their mechanism of formation, their ecological function, and their potential as beneficial pharmocological or environmentally safe agricultural products. Medical folklore is replete with descriptions of plants that heal (like aloe and ginseng) and plants that kill (like hemlock and curare);[1] the causative agent usually being a secondary metabolite. Modern medicines (like penicillin and tetracycline antibiotics) and agricultural products are often secondary metabolites or synthetic derivatives and/or analogs.

Oxidative chemical transformations of plant secondary metabolites have historically been carried out to aid in the structure elucidation of these compounds. Epoxidations with various reagents and oxidations with chromium trioxide and manganese dioxide are typical. In this dissertation, oxidative chemical transformations of plant secondary metabolites (namely, sesquiterpene lactones) have been carried out to determine the effect these changes have on the biological activities of these compounds.

Sesquiterpene lactones form one of the largest classes of plant natural products. Presently, there are well over 3200 naturally occurring sesquiterpene lactones that are known, and the number is steadily increasing.[2] Efficient procedures for the isolation of the sesquiterpene lactones used for the majority of the transformations carried out in this dissertation are described in Chapter 2.

Sesquiterpene lactones have been shown to exhibit a wide range of biological

activities. Many sesquiterpene lactones have been found to be cytotoxic and anti-cancer-active compounds in laboratory experiments. However, extensive testing by the U. S. National Cancer Institute showed these compounds were too highly toxic and thus prevented further clinical testing.[3-5] Certain sesquiterpene lactones have been found to exhibit anti-feedant properties.[6,7] Others exhibit anti-bacterial[8] and anti-fungal[9] properties and most cause allergic contact dermatitis in humans.

Some sesquiterpene lactones have been shown to exhibit molluscicidal activity against *Biomphalaria glabrata* snails.[10-12] These snails play host to miracidia (blood flukes), which in turn hatch from eggs deposited by humans suffering from schistosomiasis (or bilharzia) (Figure 1.1). Schistosomiasis is a disease which affects millions of people living in Africa, Asia, and South America. One way of attacking the disease is to eradicate the host snails. Development of an effective and environmentally safe molluscicide would certainly be beneficial to people who live in high risk areas for this disease.

Certain saponins,[10,13] napthoquinones,[14] and sesquiterpene lactones[11,12] have been shown to exhibit molluscicidal activity. Since it was shown that a hydroxylated sesquiterpene lactone exhibited the highest level of molluscicidal activity,[11,12] a series of natural and synthetic hydroxylated sesquiterpene lactones were prepared (Chapters 3 and 4). It is planned that these compounds will soon be tested for molluscicidal activity, and the structure-activity relationships derived should yield valuable information for designing an effective, selective, and environmentally safe molluscicide to help eradicate schistosomiasis.

Sesquiterpene lactones have also been shown to inhibit thiol-containing enzymes such as phosphofructokinase (PFK), glycogen synthase, DNA polymerase, and thymidylate synthase.[5] It has been proposed that the α-methylene-γ-lactone moiety of a sesquiterpene lactone acts as a receptor towards these

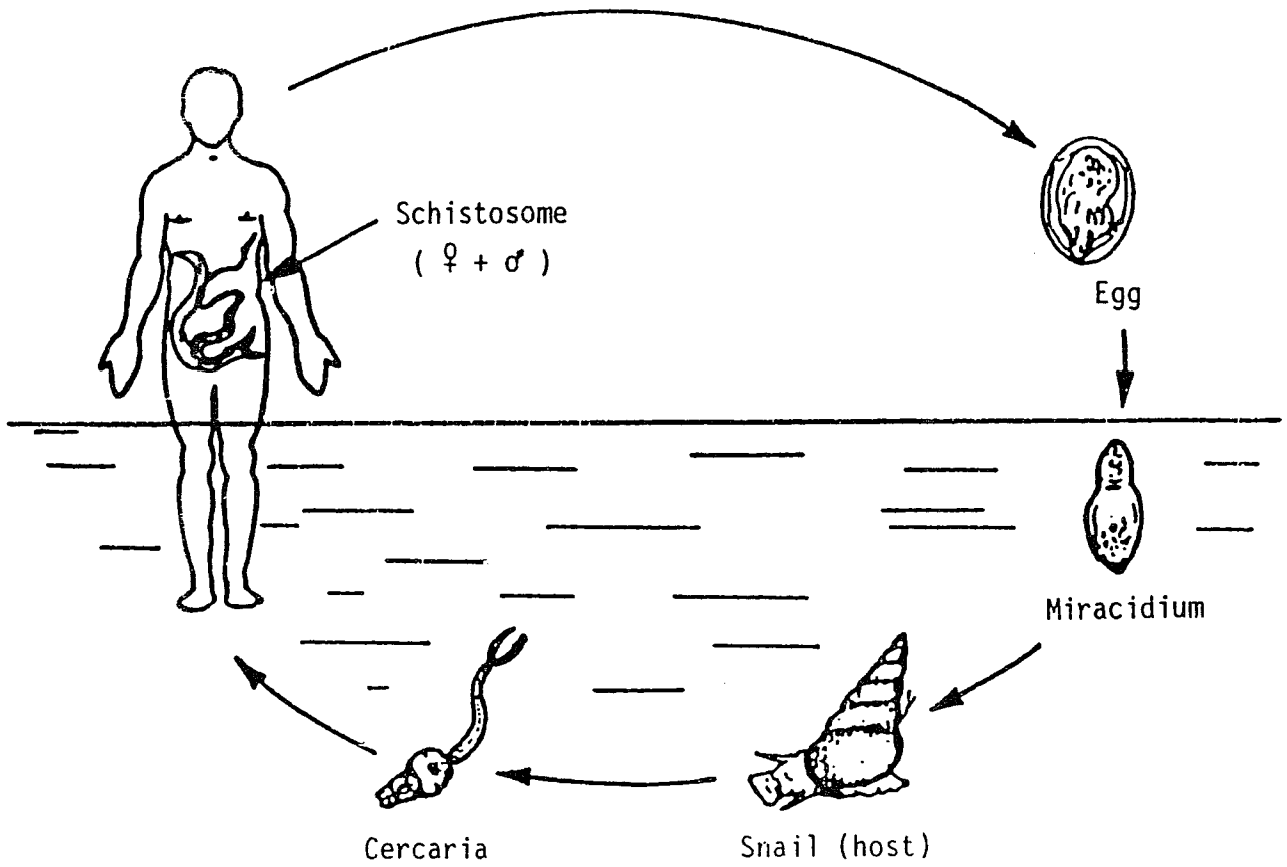


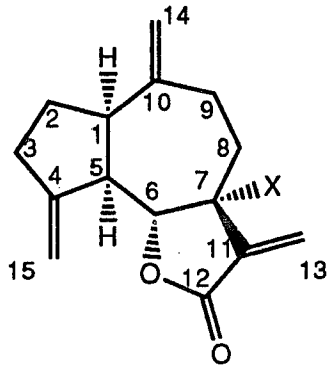
Figure 1.1 Life cycle of the schistosome. Reprinted from "Plants Used in African Traditional Medicine." by K. Hostettmann and A. Marston in Folk Medicine. The Art and the Science, R. P. Steiner, ed. 1986, ACS, Wash., D.C., p. 114.

biological nucleophiles (thiol-containing enzymes) and inhibits their activity. In the case of PFK, Vargas et al.[15] showed that while the prescence of the α-methylene-γ-lactone moiety certainly enhances the inhibition of PFK, a hydroxyl group located in proximity to the lactone functionality also enhances inhibition. For example, 7α-hydroxydehydrocostuslactone (**1**) is twenty-five times more inhibitory towards PFK than is dehydrocostuslactone (**2**) (Scheme 1.1).

Phosphofructokinase (PFK) is an enzyme which catalyzes one step in glycolysis --- the sequence of reactions that converts glucose into pyruvate with concommitant production of ATP (energy) (Scheme 1.2). PFK catalyzes the phosphorylation of fructose-6-phosphate to fructose-1,6-diphosphate (Scheme 1.3).[16] The 7-hydroxysesquiterpene lactone (**1**), which is the most effective inhibitor of PFK tested to date, might possibly be mimicking a sugar molecule (similar to fructose-6-phosphate) and initially hydrogen bond to the enzyme. Since 11-hydroxysesquiterpene lactones might equally well mimic sugar molecules, a methodology was developed and refined to synthesize a series of 11-hydroxysesquiterpene lactones (Chapter 3).

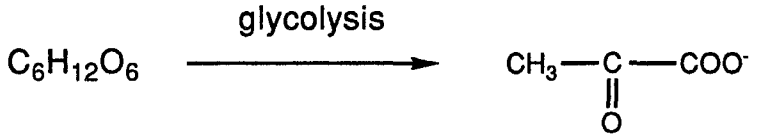
While there is no direct correlation of molluscicidal activity and PFK inhibition, it is interesting to note that the most active molluscicidal sesquiterpene lactone tested, compound (**1**), is also the most effecient inhibitor of PFK. It is our plan that the 11-hydroxysesquiterpene lactones synthesized will soon be tested for PFK inhibition.

The 11-hydroxysesquiterpene lactones might also conveniently lead to a synthesis of 7-hydroxy-α-methylene-γ-lactones by first dehydration of the 11-hydroxy-lactone to form the endocyclic double bond, second, epoxidation, and third, reductive opening of the epoxide (Scheme 1.4). The 7-hydroxy-α-methylene-γ-lactones should also be very efficient PFK inhibitors similar to compound (**1**). These transformations were met with difficulty because of limited



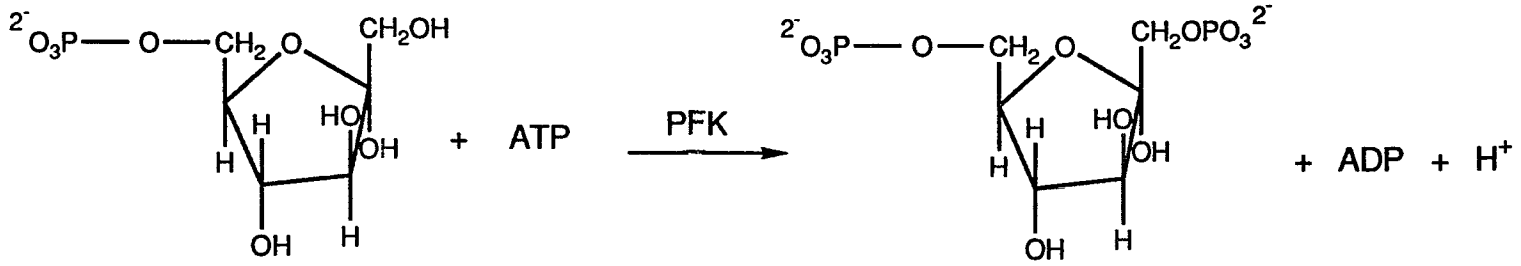
X = OH (1)
X = H (2)

Scheme 1.1

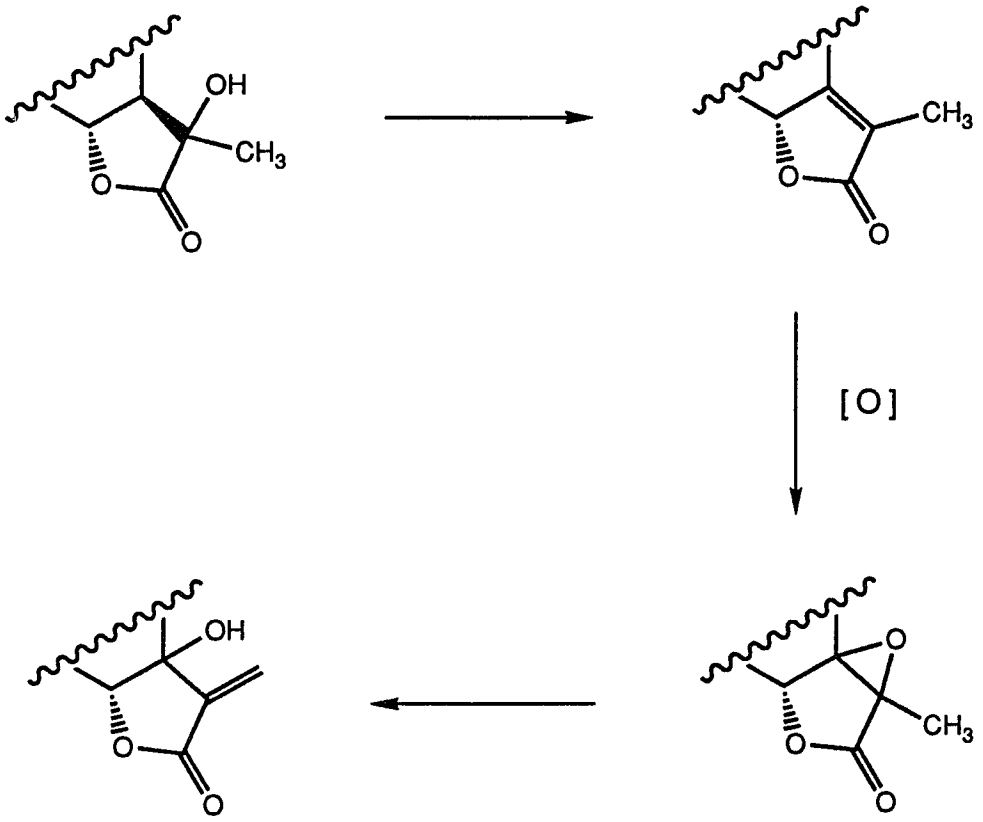


glucose pyruvate

Scheme 1.2


2- O3P — O — CH2 O CH2OH
H
HO
H
OH
OH
H
+ ATP
PFK
2- O3P — O — CH2 O CH2OPO3 2-
H
HO
H
OH
OH
H
+ ADP + H+

Scheme 1.3



Scheme 1.4

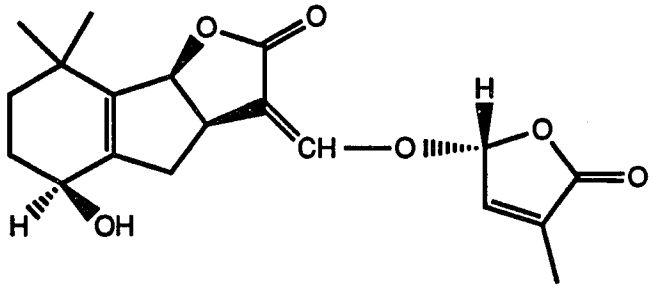
success in attempts to dehydrate the 11-hydroxy-derivatives (Chapter 5, Part C).

Certain sesquiterpene lactones have been shown to act as plant growth regulators.[5] Examples are known in which these secondary metabolites either inhibit or stimulate the growth of another plant. These results strongly suggest the ecological function of many of these compounds may be to protect the plant from attack by herbivores, fungi, or bacteria. Introduction of such a plant growth regulator to an ecosystem which requires an environmentally safe herbicide, fungicide, or bacteriocide might prove beneficial. An example of such a system would be the use of a plant growth regulator to stop the spread of witchweed.

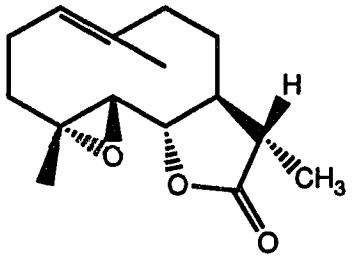
Witchweed is an obligate root parasite of major food crops including sorghum, corn, and sugarcane.[17] Dormant witchweed seeds germinate only in response to chemical signals emitted by the roots of suitable hosts.[18,19] Strigol (**3**) (Scheme 1.5), a sesquiterpene lactone, and several synthetic analogs have been shown to reduce witchweed seed populations in field tests by stimulating germination in the abscence of a suitable host, causing death of the seedling.[20,21] Dihydroparthenolide (**4**) (Scheme 1.5) and other sesquiterpene lactones have been shown to stimulate witchweed germination with comparable activity to that of strigol and its synthetic analogs.[22,23] It is hoped the testing of the synthetic and natural sesquiterpene lactones described in Chapters 3 and 4 will help define optimum structural requirements necessary for high activity and stimulation of witchweed germination.

Oxidations are important in synthetic chemistry because they are widely encountered in research and in industrial processes. Oxidations are extensively used in the laboratory synthesis of fine organic chemicals as well as the manufacture of large-volume petrochemicals. Many biological transformations (in both plants and animals) involve enzymatic oxidations.[1]

The main oxidative chemical reactions carried out and investigated in this



(3) (+)-Strigol



(4) Dihydroparthenolide

Scheme 1.5

dissertation include

a) enolate oxidations (Chapter 3),

b) allylic oxidations with singlet oxygen (Chapter 4, Part C),

c) allylic oxidations with selenium dioxide (Chapter 4, Parts A,B, and D), and

d) epoxidation of alkenes (Chapter 3, Part A, Chapter 4, Part D, and Chapter 5, Part B).

The synthetic problem of enolate hydroxylation with molecular oxygen has seen limited use in the literature.[24-28] Hydroperoxides are the initial products of such oxidations, but when a reducing agent like dimethylsulfoxide or a trialkyl phosphite is present, the corresponding alcohol is isolated.[29] Use of lithium diisopropylamide (LDA) as the base to generate the enolate anion at the same time generates diisopropylamine which is also capable of reducing hydroperoxides to alcohols.[30] Thus, when LDA-generated enolates are reacted with oxygen, alcohol products are isolated. The enolate oxidation of sesquiterpene lactones with oxygen was first used by Collado et al.[31] in the synthesis of subexpimatin C. This reaction was used extensively to generate a series of 11-hydroxysesquiterpene lactones which are potential molluscicidal compounds and may be effective inhibitors of the glycolytic enzyme phosphofructokinase.

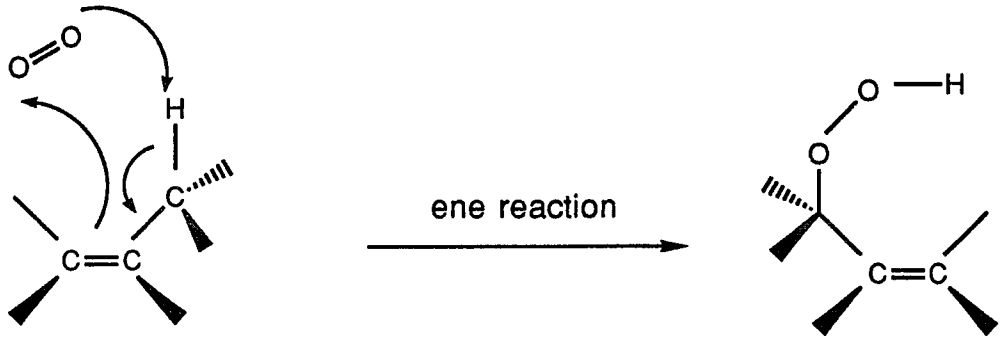
Other enolate hydroxylation methods reported in the literature include oxidation of enolates with the molybdenum peroxide reagent MoO_5·pyridine·HMPA (MoOPH)[32,33] and with (camphorylsulfonyl)oxaziridines.[34-36] The former reagent is superior to oxygen because it generates higher yields of α-hydroxy-carbonyl compounds and affords no products of oxidative C-C cleavage; however, preparation of the reagent is not trivial and the reagent itself is light sensitive and may be shock sensitive. The molybdenum peroxide reagent was not used for any of the enolate oxidations carried out in this dissertation. The latter reagent

(oxaziridines) is also superior to oxygen for the same reasons, but additionally, the oxaziridines are more stable, they are commercially available, and they can be recovered and regenerated. Enolate oxidations with oxaziridines were carried out in Chapter 3, Part B.

Allylic positions of alkenes can be oxidized to generate unsaturated hydroperoxides, alcohols, and carbonyl compounds. Unsaturated hydroperoxides are formed from the reaction of alkenes with singlet oxygen.[37] Singlet oxygen is usually generated by irradiation of solutions of oxygen in the presence of a photosensitizer. An ene reaction occurs in which an allylic proton is abstracted along with migration of the carbon-carbon double bond (Scheme 1.6). The synthesis of peroxydihydroparthenolide (Chapter 4, Part C) was achieved using singlet oxygen.

Allylic oxidations have also been achieved using selenium dioxide (SeO_2). Allylic alcohols are generated from alkenes via an ene reaction followed by a 2,3-sigmatropic rearrangement. Umbreit and Sharpless[38] reported that only catalytic amounts of SeO_2 are necessary when the reaction is carried out in the prescence of tert-butylhydroperoxide. This reduces the amount of SeO_2 necessary to carry out these transformations and eliminates complications from production of reduced forms of toxic selenium. Haruna and Ito[39] were the first to carry out this reaction on sesquiterpene lactones. Allylic oxidation with SeO_2 was used in the synthesis of two naturally occurring sesquiterpene lactones, 11β,15-dihydroxysaussurea lactone and 15-hydroxydihydrocostunolide (Chapter 4, Parts A and B). The latter synthesis involves a novel method for the introduction of a hydroxy group into the 15-position of a germacranolide. Allylic oxidation with SeO_2 was also used in an attempt to carry out a biomimetic transformation of one skeletal type of germacranolide into another (Chapter 4, Part D).

Oxidations of allylic methylene groups to carbonyl groups have been carried out



Scheme 1.6

using chromic oxide,[40,41] its complexes with pyridine,[42,43] and with tert-butylhydroperoxide in the prescence of a chromium carbonyl catalyst.[44] Attempts were made to carry out these transformations on sesquiterpene lactones (Chapter 5, Part B).

Various methods of epoxidizing alkenes are known,[45] with the most common reagent being peroxyacids. The epoxidation of the 1,10-double bond of various germacrolides was carried out (Chapter 3, Part A and Chapter 4, Part D) using meta-chloroperoxybenzoic acid in the prescence of a buffer to prevent transannular cyclizations.[46]

Chapter 5 is a collection of miscellaneous transformations of sesquiterpene lactones, which includes both reductions and oxidations.

References

1. Hebert, R.B. The Biosynthesis of Secondary Metabolites, 2nd edition, **1989**, New York, Chapman and Hall.
2. Vasquez, M. Dissertation. "Structure Elucidation of Secondary Metabolites From *Rudbeckia* Species By Spectroscopic Techniques and Review of Sesquiterpene Lactones." Louisiana State University, **1989**.
3. Cassady, J.M.; Suffness, M. "Terpenoid Anti-tumor Agents" in Anticancer Agents Based on Natural Products Models, **1980**, J.M.Cassady, J.D.Douros, eds., Academic Press, London, pp.201-69.
4. Misra, R.; Pandey, R. C. "Cytotoxic and Antitumor Terpenoids" in Antitumor Compounds of Natural Origin. Chemistry and Biochemistry, **1981**, A. Aszalos, ed., CRC Press, Boca Raton, Vol. 2, pp. 145-92.
5. Picman, A.K. *Biochem. System. Ecol.* **1986**, *14*, 255-81.
6. Nawrot, J.; Bloszyk, E.; Harrantha, J.; Novotny, L.; Drozdz, B. *Acta. Entomol. Bohemoslov.* **1986**, *83(5)*, 327-35.
7. Burnett, W.C., Jr.; Jones, S.B., Jr.; Mabry, T.J.; Padolina, W.G. *Biochem. System. Ecol.* **1974**, *2*, 25-9.
8. Picman, A. K.; Towers, G. H. N. *Biochem. System. Ecol.* **1983**, *11*, 321-7.
9. Gennari, M.; Abbattista Gentile, I.; Cugudda, L. Z. *Pflanzenkrankh. Pflanzenschutz* **1987**, *94(1)*, 68-73.
10. Marston, A.; Hostettmann, K. *Phytochemistry* **1985**, *24*, 639-52.
11. Fronczek, F.R.; Vargas, D.; Fischer, N.H.; Hostettmann, K. *J. Nat. Prod.* **1984**, *47(6)*, 1036-9.
12. Vargas, D.; Fronczek, F.R.; Fischer, N.H.; Hostettmann, K. *J. Nat. Prod.*

1986, *49*, 133-8.

13. Gafner, F.; Msonthi, J.D.; Hostettmann, K. *Helv. Chim. Acta.* **1985**, *68*, 555-8.
14. Marston, A.; Msonthi, J.D.; Hostettmann, K. *Planta Med.* **1984**, *50*, 279.
15. Vargas, D.; Younathan, E.S.; Fischer, N.H., unpublished results.
16. Stryer, L. Biochemistry, 2nd edition, **1981**, W.H. Freeman and Co., New York, pp. 255-82.
17. Shaw, W.C.; Sheppard, D.R.; Robinson, E.L.; Sand, P.F. *Weeds* **1962**, *10*, 182.
18. Chang, M.; Netzly, D.H.; Butler, L.G.; Lynn, D.G. *J. Am. Chem. Soc.* **1986**, *108*, 7858-60.
19. Netzly, D.H.; Riopel, J.L. Ejeta, G.; Butler, L. G. *Weed Sci.* **1988**, *36*, 441.
20. Johnson, A.W.; Rosenberry, G.; Parker, C. *Weed Res.* **1976**, *16*, 223.
21. Pepperman, A. B.; Connick, W.J., Jr.; Vail, S.L.; Worsham, A. D.; Paulista, A. D.; Moreland, D.E. *Weed Sci.* **1982**, *30*, 561.
22. Fischer, N.H.; Weidenhamer, J. D.; Bradow, J. M. *Phytochemistry* **1989**, *28(9)*, 2315-7.
23. Fischer, N.H.; Weidenhamer, J.D.; Bradow, J. M. *Phytochemistry* **1990**, *29*, 2479-83.
24. Gardner, J. N.; Popper, T.L.; Carlon, F.E.; Gnoj, O.; Herzog, H.L. *J. Org. Chem.* **1968**, *33*, 3695-9.
25. Corey, E.J.; Engley, H.E. *J. Am. Chem. Soc.* **1975**, *97*, 6908-9.
26. Plattner, J.J.; Gless, R.D.; Rapoport, H. *J. Am. Chem. Soc.* **1972**, *94*, 8613-15.
27. Volkmann, R.; Danishefsky, S.; Eggler, J.; Solomon, D.M. *J. Am. Chem.*

Soc. **1971**, *93*, 5575-6.

28. Buchi, G.; Matsumoto, K.E.; Nishimura, H. *J. Am. Chem. Soc.* **1971**, *93*, 3299-3301.
29. Gardner, J.N.; Carlon, F.E.; Gnoj, O. *J. Org. Chem.* **1968**, *33*, 3294-7.
30. Biloski, A.; Ganem, B. *Synthesis* **1983**, *7*, 537-8.
31. Collado, I.G.; Macias, F.A.; Massanet, G. M.; Molinillo, J.M.G.; R.-Luis, F. *J. Org. Chem.* **1987**, *52*, 3323-6.
32. Vedejs, E. *J. Am. Chem. Soc.* **1974**, *96*, 5944-6.
33. Vedejs, E.; Engler, D.A.; Telschow, J.E. *J. Org. Chem.* **1978**, *43*, 188-96.
34. Towson, J. C.; Weismiller, M.C.; Lal, G.S.; Sheppard, A.C.; Davis, F.A. *Org. Synth.* **1990**, *69*, 158-67.
35. Davis, F.A.; Haque, M.S. *J. Org. Chem.* **1986**, *51*, 4085-7.
36. Davis, F.A.; Haque, M.S.; Ulatowski, T.G.; Towson, J.C. *J. Org. Chem.* **1986**, *51*, 2402-4.
37. Wasserman, H.H.; Ives, J.L. *Tetrahedron* **1980**, *37*, 1825-52.
38. Umbreit, M. A.; Sharpless, K.B. *J. Am. Chem. Soc.* **1977**, *99*, 5526-8.
39. Haruna, M.; Ito, K. *J.C.S. Chem. Comm.* **1981**, 483-5.
40. Rosenthal, D.; Grabowich, P.; Sabo, E.F.; Fried, J. *J. Am. Chem. Soc.* **1963**, *85*, 3971-9.
41. Nakayama, M.; Shinke, S.; Matsushita, Y.; Ohira, S.; Hayashi, S. *Bull. Chem. Soc. Jpn.* **1979**, *52* 184-5.
42. Dauben, W.G.; Lorber, M.; Fullerton, D.S. *J. Org. Chem.* **1969**, *34*, 3587-92.
43. Parish, E. J.; Chitrakorn, S.; Wei, T.-Y. *Synth. Commun.* **1986**, *16*, 1371-5.

44. Pearson, A.J.; Chen, Y.-S.; Hsu, S.-Y.; Ray, T. *Tetrahedron Lett.* **1984**, *25*, 1235-8.

45. Hudlicky, M. Oxidations in Organic Chemistry. American Chemical Society, Wash., D.C. **1990**, pp. 60-4.

46. Rodriguez, A.A.S.; Garcia, M.; Rabi, J. *Phytochemistry* **1978**, 953-4.

Chapter 2. Isolation, Separation, and Purification of Sesquiterpene Lactones.

Introduction

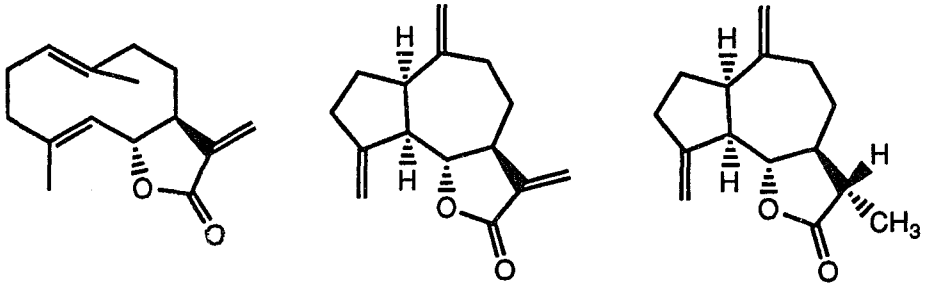
The preferred method for large-scale isolations of sesquiterpene lactones from plants[1] was introduced by Herz and Hogenauer in 1961.[2] Application of the Herz and Hogenauer procedure removes chlorophyll and plant phenolics from crude plant extracts by precipitation with lead(II) acetate, providing a crude terpenoid sample which can be further purified by various chromatographic methods. The Herz and Hogenauer procedure was used for the large-scale isolation of dihydroparthenolide (Chapt. 2, Part B) necessary for subsequent synthetic work.

Various chromatographic methods have been used for the separation of sesquiterpene lactones from crude terpenoid extracts including column chromatography, preparative thin-layer chromatography (TLC), high performance liquid chromatography (HPLC), gas-liquid chromatography (GLC)[1], and supercritical fluid chromatography (SFC).[3] Vacuum liquid chromatography (VLC)[4,5] is a very simple, efficient, and inexpensive separation technique which has also been used successfully for the separation of sesquiterpene lactones from crude terpenoid extracts.[6] A modified suction filtration set-up is used for VLC in which TLC-grade silica gel is packed under vacuum in a sintered-glass Buchner filter funnel. The large-scale separations (1-10g. of crude) of costunolide and dehydrocostuslactone from Costus Resin (Chapt. 2 Part A) and dihydroparthenolide from *Ambrosia artemisiifolia* (Chapt. 2, Part B) were efficiently achieved by VLC.

Part A. Separation and Purification of Costunolide and Dehydrocostuslactone From Costus Resinoid.

Introduction

The essential oil of the costus plant, (*Saussurea lappa* Clark) obtained commercially as Costus Resinoid (Pierre Chauvet, S.A., France), was highly valued in the 1950's as a blending agent in the perfume industry.[7] The resin contains the following constituents: costunolide (**1**), dehydrocostuslactone (**2**), and dihydrodehydrocostuslactone (**3**).



1 2 3

Dehydrocostuslactone (**2**) was first isolated from costus oil by Romanuk et al. in 1958.[8] Costunolide (**1**) was first isolated from the costus plant by Bhattacharyya et al. after solvent extraction.[9,10,11] In 1977, Grieco and Nishizawa reported the total synthesis of costunolide (**1**) starting from α-santonin.[12]

HPLC separation of **1** and **2** had been previously achieved[13] using a PrePAK-500/silica column, eluting with petroleum ether/ether (8:1) at a flow rate of 0.5 L./min. Below, the isolation of costunolide (**1**) and dehydrocostuslactone (**2**) from Costus Resinoid is described using the more efficient method of vacuum liquid chromatography (VLC).

The more polar constituents of the Costus Resinoid can be separated by VLC on

a 5cm. diameter by 5cm. high column prepared with TLC grade silica gel (MN Kieselgel G) adsorbent which was packed under vacuum. The crude resin (5-10grams) was dissolved in a minimum amount of dichloromethane (DCM) and mixed with an equivalent weight of silica gel. After evaporation of the solvent, the dried powder was packed onto the top of the column. The crude resin was eluted first with DCM (4 x 100ml), followed by ethyl acetate (EtOAc) (4 x 100ml). Costunolide (**1**) and dehydrocostuslactone (**2**) are the major constituents of the combined DCM fractions. By thin-layer chromatography (TLC) (silica gel, eluting with DCM), after spraying with $CoCl_2/H_2SO_4$ and heating the plate[1], **2** appears as a pink spot (R_f = 0.80), and **1** appears as a grey spot (R_f = 0.75) with partial overlap.

Separation of **1** and **2** requires a second VLC separation using the residue of the DCM fraction. Exhaustive elution with hexane (10-15 fractions of 100ml) is followed by elution with hexane/DCM mixtures, in which the DCM content is increased by 10% increments with each fraction. The less polar fractions (hexane) contain relatively pure dehydrocostus lactone (**2**) and the more polar fractions are very rich in costunolide (**1**). Fractions should be monitored by TLC. Costunolide (**1**) crystallizes from the more polar fractions; a seed crystal may be necessary to induce crystallization. Costunolide crystals are washed with cold hexane to remove some of the yellowish, gummy impurities and can be recrystallized from diethyl ether. Costunolide crystals should be stored under an inert solvent (like hexane) in a capped vial, and placed in a freezer to prevent polymerization.

Experimental Section

^1H and ^{13}C NMR spectra were recorded on a Bruker AC-200 spectrometer in

$CDCl_3$ using $SiMe_4$ as an internal standard. Mass spectra were recorded on a HP5985 spectrometer. Infrared spectra were recorded either on a Perkin-Elmer 257 or 1760x spectrometer.

Costunolide (1)[14-17] Experimental melting point 109-114°C (Lit. m.p.[9] 107°C) IR 1761, 1660cm^{-1}; 200MHz ^1H NMR (Fig. 2.1): δ 6.25 (d,1H,C_{13}-Hb,J=3Hz), 5.51 (d,1H,C_{13}-Ha,J=3Hz), 4.85 (m,1H,C_1-H), 4.73 (d,1H,C_5-H,J=10Hz), 4.55 (dd,1H,C_6-H,J=9Hz), 1.68 (s,3H,C_{15}-CH_3), 1.41 (s,3H,C_{14}-CH_3); 50.3MHz ^{13}C NMR (Fig. 2.2), assignments:[18] δ 170.4 (C-12), 141.4 (C-11), 140.1 (C-10), 136.9 (C-4), 127.3 (C-1), 127.0 (C-5), 119.5 (C-13), 81.9 (C-6), 50.4 (C-7), 40.9 (C-3), 39.4 (C-9), 28.0 (C-8), 26.1 (C-2), 17.3 (C-15), 16.0 (C-14); MS *m/z* (relative intensity) 232 (M^+) (4.9), 217 (M-15^+) (5.1), 204 (M-28^+) (0.9), 189 (M-43^+) (1.8), 175 (M-57^+) (4.5).

Dehydrocostuslactone[19-22] **(2)** IR 1770, 1638cm^{-1}; 200MHz ^1H NMR (Fig. 2.3): δ 6.17 (d,1H,C_{13}-Hb,J=3Hz), 5.46 (d,1H,C_{13}-Ha,J=3Hz), 5.23 (d,1H,C_{15}-H,J=2Hz), 5.02 (d,1H,C_{15}-H, J=2Hz), 4.86 (s,1H,C_{14}-H), 4.74 (s,1H,C_{14}-H), 3.93 (dd,1H,C_6-H,J=9Hz); 50.3MHz ^{13}C NMR (Fig. 2.4), assignments:[18] δ 169.6 (C–12), 151.0 (C-4), 148.9 (C-10), 139.4 (C-11), 119.6 (C-13), 112.0 (C-14), 108.9 (C-15), 84.8 (C-6), 51.6 (C-5), 47.1 (C-1), 44.7 (C-7), 36.0 (C-9), 32.3 (C-3), 30.6 (C-8), 29.9 (C-2); MS *m/z* (relative intensity) 230 (M^+) (6.1), 187 (M-43^+) (1.7).

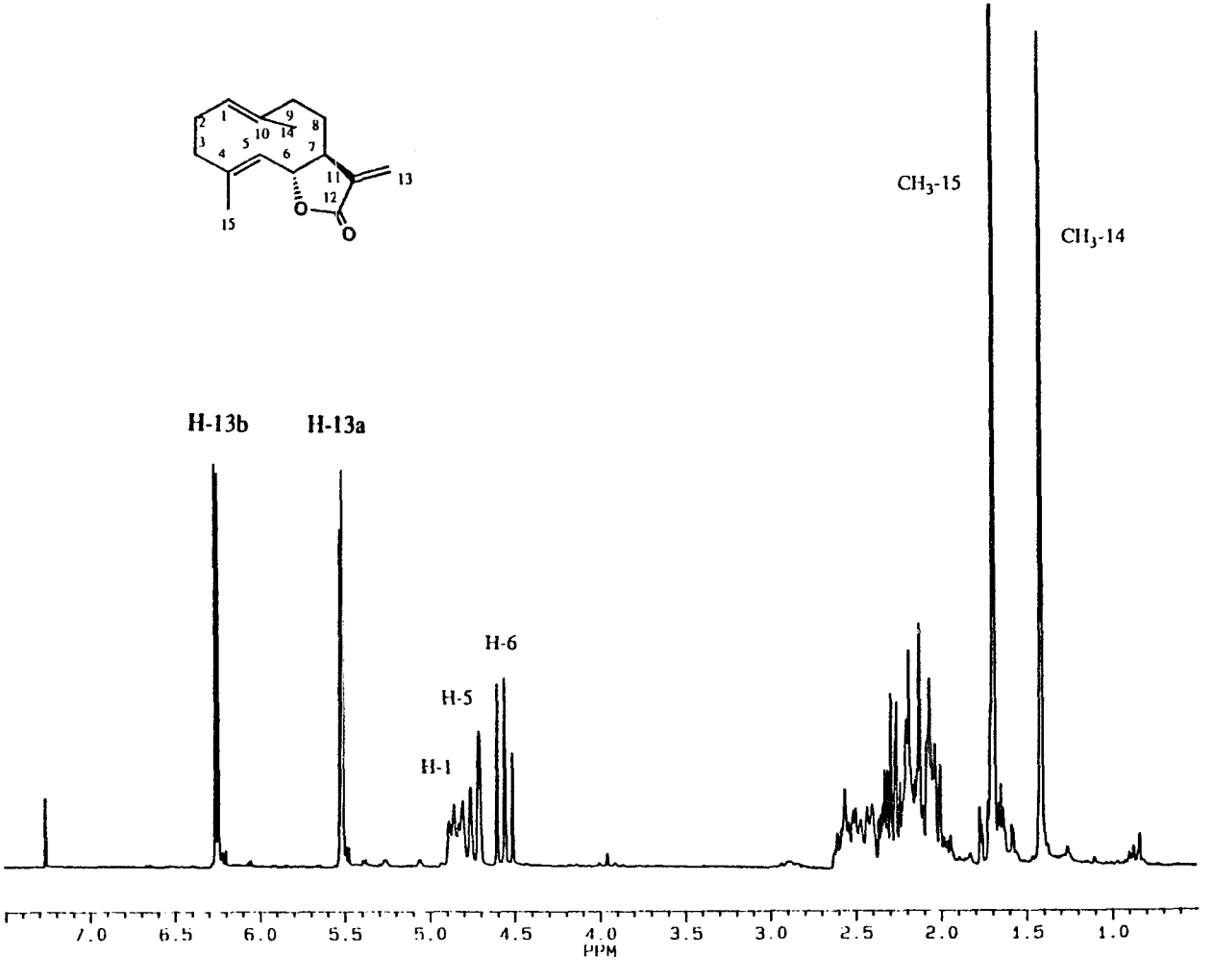


Figure 2.1. ^1H NMR spectrum of costunolide **(1)** in $CDCl_3$.

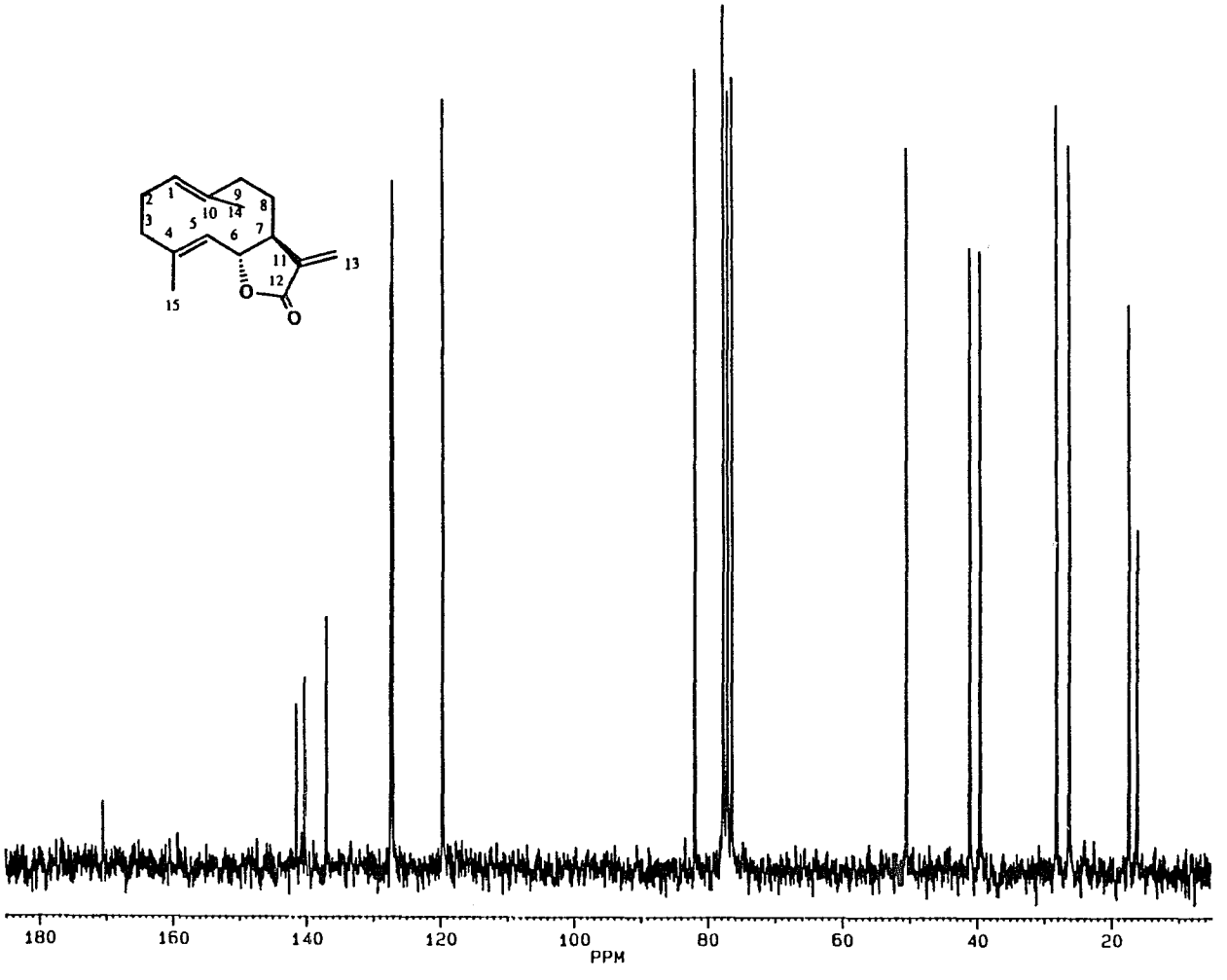


Figure 2.2. ^{13}C NMR spectrum of costunolide **(1)** in $CDCl_3$.

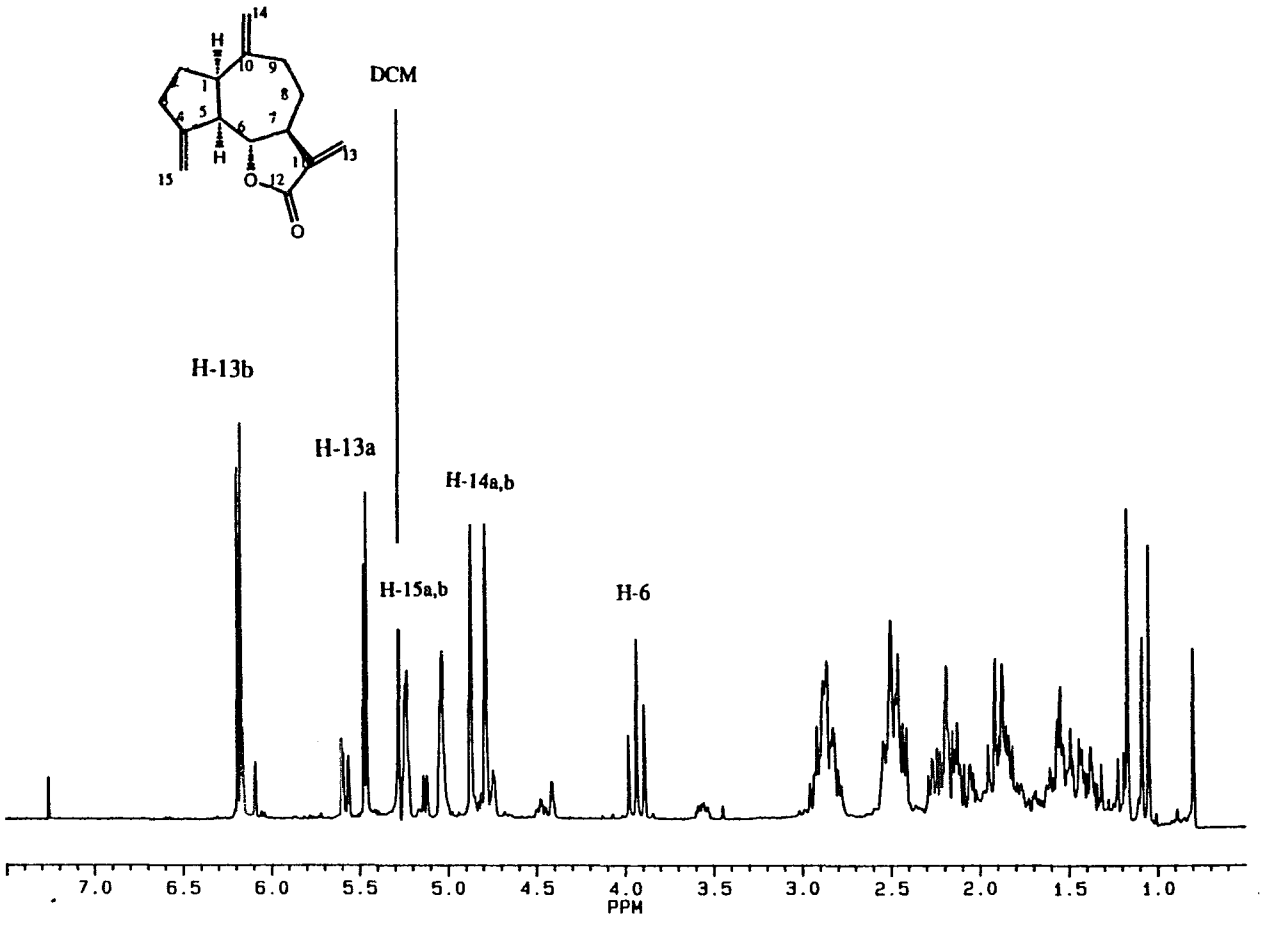


Figure 2.3. ^{1}H NMR spectrum of dehydrocostus lactone **(2)** in $CDCl_3$.

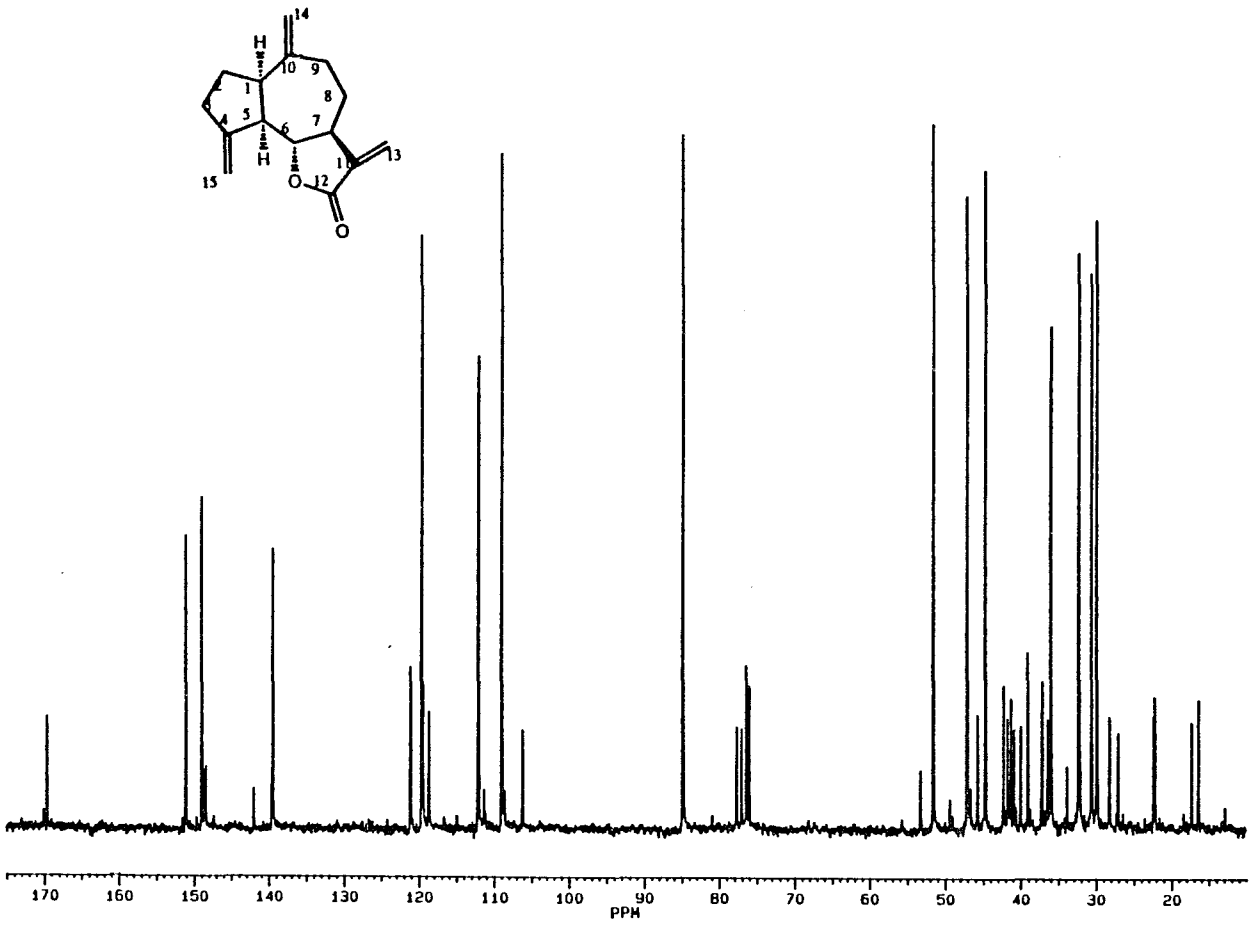


Figure 2.4. ^{13}C NMR spectrum of dehydrocostus lactone **(2)** in $CDCl_3$.

References

1. Fischer, N.H. Sesquiterpene Lactones. in Methods in Plant Biochemistry. The Terpenoids. Vol. 7, Academic Press, London, **1991**, in press.
2. Herz, W.; Hogenauer, G.C. *J. Org. Chem.* **1961**, *26*, 5011-3.
3. Crow, J.A.; Foley, J.P. *Anal. Chem.* **1990**, *62*, 378-86.
4. Coll, J. C.; Bowdon, B.F. *J. Nat. Prod.* **1986**, *49*, 934-6.
5. Pelletier, S.W.; Chokshi, H.P.; Desai, H.K. *J. Nat. Prod.* **1986**, *49*, 892-900.
6. Quijano, L., private communication.
7. Guenther, E. *Essential Oils* **1952**, *5*, 446.
8. Romanuk, M.; Herout, V.; Sorm, F. *Coll. Czech. Chem. Commun.* **1958**, *23*, 2188-94.
9. Rao, A.S.; Kelkar, G.R.; Bhattacharyya, S.C. *Tetrahedron* **1960**, *9*, 275-83.
10. Rao, A.S.; Kelkar, G.R.; Bhattacharyya, S.C. *Chem. and Ind.* **1958**, 1359-60.
11. Bhattacharyya, S.C.; Kelkar, G.R.; Rao, A.S. *Chem. and Ind.* **1959**, 1069.
12. Grieco, P.A.; Nishizawa, M. *J. Org. Chem.* **1977**, *42*, 1717-20.
13. Lee, Ihl Young. Dissertation. "New Sesquiterpene Lactones From The Genera *Calea* And *Berlandiera* (Asteraceae) And The Fragmentation Reactions of 1,3-Dihydroeudesmanolide Derivatives." Louisiana State University, **1983**, 104-7.
14. El-Feraly, F.S.; Chan, Y. *J. Pharm. Sci.* **1978**, *67*, 347-50.

15. Rodrigues, A.A.S.; Garcia, M.; Rabi, J.A. *Phytochemistry* **1978**, *17*, 953-4.

16. Kalsi, P.S.; Khurana, S.; Talwar, K.K. *Phytochemistry* **1985**, *24*, 103-9.

17. Sathe, R.N.; Kulkarni, G.R.; Kelkar, G.R.; Das, K.G. *Org. Mass Spect.* **1969**, *2*, 935-45.

18. DeLuengo, D.H.; Miski, M.; Gage, D.A.; Mabry, T.J. *Phytochemistry* **1986**, *25*, 1917-22.

19. Semmler, F.W.; Feldstein, J. *Ber. Dt. Chem. Ges.* **1914**, *47*, 2433-7.

20. Romanuk, M.; Herout, V.; Sorm, F. *Colln. Trav. Chim. Tchecosl.* **1956**, *21*, 894-900.

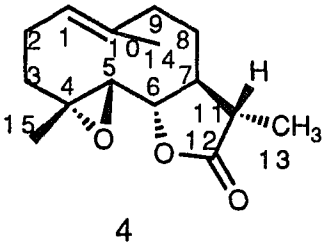
21. Kalsi, P.S.; Vij, V. K.; Singh, O.S.; Wadia, M. S. *Phytochemistry* **1977**, *16*, 784-86.

22. Fronczek, F.R.; Vargas, D.; Parodi, F.; Fischer, N.H. *Acta. Cryst.* **1989**, *C45*, 1829-31.

Part B. Isolation, Separation, and Purification of Dihydroparthenolide and Psilostachyin A From *Ambrosia artemisiifolia* .

Introduction

Dihydroparthenolide (DHP) (**4**), a naturally occurring sesquiterpene lactone, has been isolated from *Ambrosia artemisiifolia*,[1,2] the common ragweed, which grows locally in Baton Rouge, Louisiana. DHP has been shown to stimulate the germination of witchweed which is a root parasite of major food crops like sorghum, corn, and sugarcane.[3] Below is a description of an efficient method for large-scale isolation, separation, and purification of DHP from ragweed which was necessary in order to carry out desired synthetic transformations.



4

A bulk collection of *Ambrosia artemisiifolia* was made in April, 1989 at the Woodgate subdivision (off of Highland Road) in East Baton Rouge Parish, Louisiana (Fischer, Pentes, Weidenhamer # 378). Dry ground aerial plant parts (2.5kg) were extracted with dichloromethane (DCM) by soaking for 24hrs. (3x) yielding 65g (2.6% of plant dry weight) of a dark green syrup. The presence of DHP in the crude syrup can best be detected by thin-layer chromatography (TLC) analysis using silica gel as adsorbent and eluting with chloroform/acetone (3:1). After spraying the TLC plate with $CoCl_2/H_2SO_4$ and heating the plate[4], DHP appears as an intense red spot with R_f=0.87. Detection of DHP in the crude syrup by ^1H NMR analysis is less clear. Usually, the most distinct ^1H NMR signals of

DHP (H-1, H-5, H-6, CH_3-13, CH_3-14, CH_3-15) are broadened in the crude syrup or they overlap with other signals making detection by NMR quite difficult.

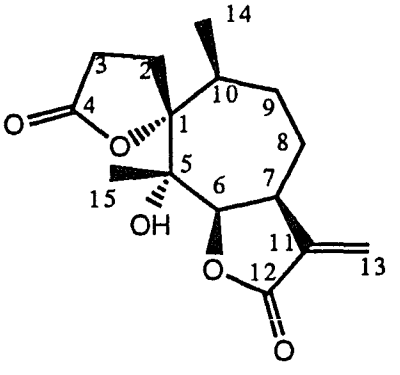
The crude DCM extract syrup was subjected to the Herz and Hogenauer procedure[5], which precipitates most of the fats, fatty acids, phenolic compounds, and chlorophyll, yielding 13g of crude terpenoid extract. Dissolving the 13g of terpenoid extract in isopropanol, adding a seed crystal, and placing the solution in the freezer (-15°C) did not result in crystallization of DHP within 7 days.

The crude DCM extract syrup (1g) was dissolved in 95% ethanol (100ml) and an equal volume of 5% aqueous $Pb(OAc)_2$ solution was added. The solution was filtered over a pad of Celite and most of the ethanol was evaporated from the filtrate. The aqueous solution was then extracted with DCM (50ml x10).The DCM solution was dried over anhydrous Na_2SO_4 and the solvent was evaporated.

The components of this crude terpenoid mixture were separated by vacuum liquid chromatography (VLC).[6,7] A 5cm. high by 5cm. diameter column of silica gel (MN Kieselgel G) was used as adsorbent. The column was first eluted with hexane/DCM (3:2; 5 x 100ml) followed by an increasingly polar mobile phase made by increasing the amount of DCM. DHP crystallized from the early fractions: seed crystals were sometimes necessary to induce crystallization. Crude DHP crystals were recrystallized from isopropanol. From 65g. of crude plant extract, 1.7g (0.068% of dry plant material) of DHP were isolated. DHP crystals are stable to both air and light and need not be kept under an inert solvent in the freezer. Some DHP (400mg) crystallized from the concentrated aqueous solution before extraction with DCM.

A prior bulk collection of *Ambrosia artemisiifolia* was made in July, 1988 near Perkins Road in East Baton Rouge Parish, Louisiana. 200gm (4% of plant dry weight) of crude syrup was isolated from the DCM extraction of 5kg of dry,

ground plant material. Dissolving some of this crude DCM extract syrup in isopropanol, adding a seed crystal, and placing this solution in the freezer did not result in crystallization of DHP even after 7 days. Also, no DHP crystallized from fractions collected off of a silica gel VLC column of the crude DCM extract syrup when eluted with chloroform/acetone (3:1). Crude extract (40g.) was subjected to the Herz and Hogenauer procedure[5], yielding a terpenoid crude which partially crystallized. When it was attempted to dissolve this partially crystalline syrup in isopropanol, some material was insoluble and was separated by gravity filtration. This white solid residue (150mg) was analyzed by ^{1}H NMR, MS, FTIR and single crystal X-ray diffraction and determined to be identical to psilostachyin A (**5**)[8]. The remainder of the crude extract (130g) was subjected to the Herz and Hogenauer procedure[5] followed by VLC in which 1.1g (0.035% of dry plant material) of DHP was isolated.



5

The structure of DHP was first established using low-field NMR data and degradation experiments.[9] High-field NMR spectral studies of DHP have also been done including 2D-$^{13}C^{13}C$ shift correlation spectrum, 2D-$^{1}H^{13}C$ correlation spectrum, and various NOEDIFF experiments.[1] The NMR data indicate that DHP adopts a solute conformation such that the two methyl groups are β- oriented and

the 1,10-double bond and the 4,5-epoxide are trans and crossed, as in the conformation of parthenolide which was established by X-ray analysis.[10]

Experimental Section

^1H and ^{13}C NMR spectra were recorded on a Bruker-AC200 spectrometer in $CDCl_3$ using $SiMe_4$ as an internal standard. Mass spectra were recorded on a HP5985 spectrometer. Infrared spectra were recorded either on a Perkin-Elmer 257 or 1760x spectrometer.

Dihydroparthenolide (4) Experimental melting point 131-133°C (lit. m.p.[1] 137°C) IR 1776cm^{-1}; 200MHz ^1H NMR (Fig. 2.5): δ 3.80 (dd,1H,C_6-H,J=9Hz), 2.69 (d,1H,C_5-H,J=9Hz), 1.67 (s,3H,C_{14}-CH_3), 1.29 (s,3H,C_{15}-CH_3), 1.27 (d,3H,C_{13}-CH_3),J=7Hz); 50.3MHz ^{13}C NMR (Fig. 2.6), assignments:[1] δ 177.3 (C-12), 134.4 (C-10), 125.1 (C-1), 82.1 (C-6), 66.3 (C-5), 61.4 (C-4), 51.9 (C-7), 42.4 (C-11), 41.1 (C-9), 36.6 (C-3), 29.7 (C-8), 24.0 (C-2), 17.1 (C-15), 16.8 (C-14), 13.2 (C-13); MS *m/z* (relative intensity) 250 (M^+) (3.0), 235 (M-15$^+$) (8.7), 232 (M-18$^+$) (0.2), 207 (M-43$^+$) (4.6).

Psilostachyin A (5)[11] Experimental melting point 217-221°C (lit. m.p.[10] 215°C) IR 3493, 1763cm^{-1}; 200MHz ^1H NMR (Fig. 2.7): δ 6.25 (d,1H,C_{13}-Hb,J=3Hz), 5.54 (d,1H,C_{13}-Ha,J=3Hz), 4.95 (d,1H,C_6-H,J=10Hz), 3.42 (m,1H,C_7-H), 1.21 (s,3H,C_{15}-CH_3), 1.02 (d,3H,C_{14}-CH_3,J=7Hz); 50.3MHz ^{13}C NMR (Fig. 2.8), assignments:[12] δ 177.3 (C-4), 169.7 (C-12), 138.9 (C-11), 121.7 (C-13), 93.7 (C-5), 83.4 (C-6), 79.4 (C-1), 41.5 (C-7), 40.1 (C-10), 30.1 (C-8), 27.5 (C-3), 26.8 (C-9), 24.3 (C-2), 21.5 (C-15), 15.0 (C-14); MS *m/z*

(relative intensity) 280 (M^+) (0.9), 262 ($M-18^+$) (3.2), 247 ($M-33^+$) (1.2), 244 M-36^+) (1.1), 234 ($M-46^+$) (3.4), 219 ($M-61^+$) (18.9). The molecular structure of **5** is shown in Figure 2.9.

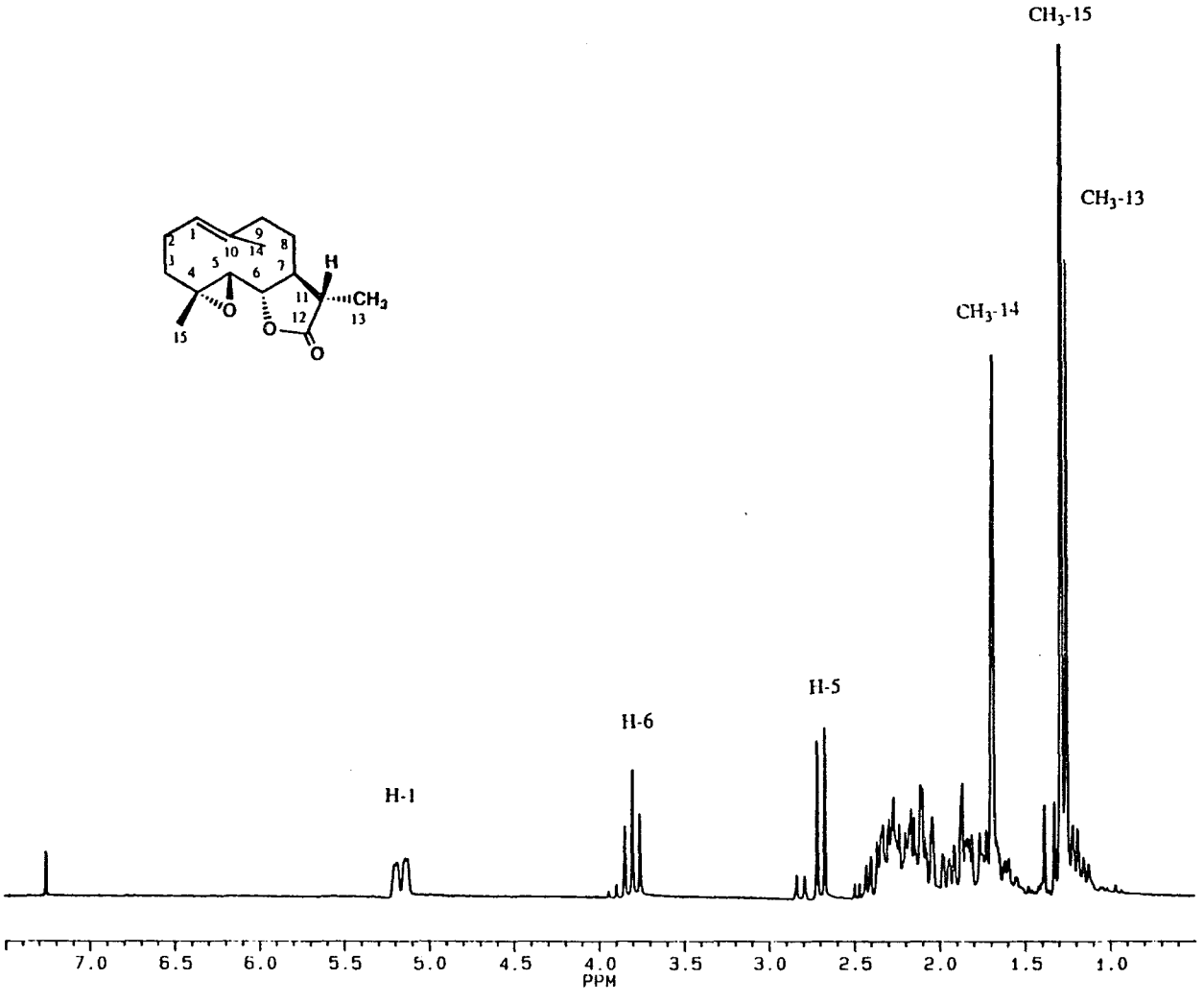


Figure 2.5. ^1H NMR spectrum of dihydroparthenolide **(4)** in $CDCl_3$.

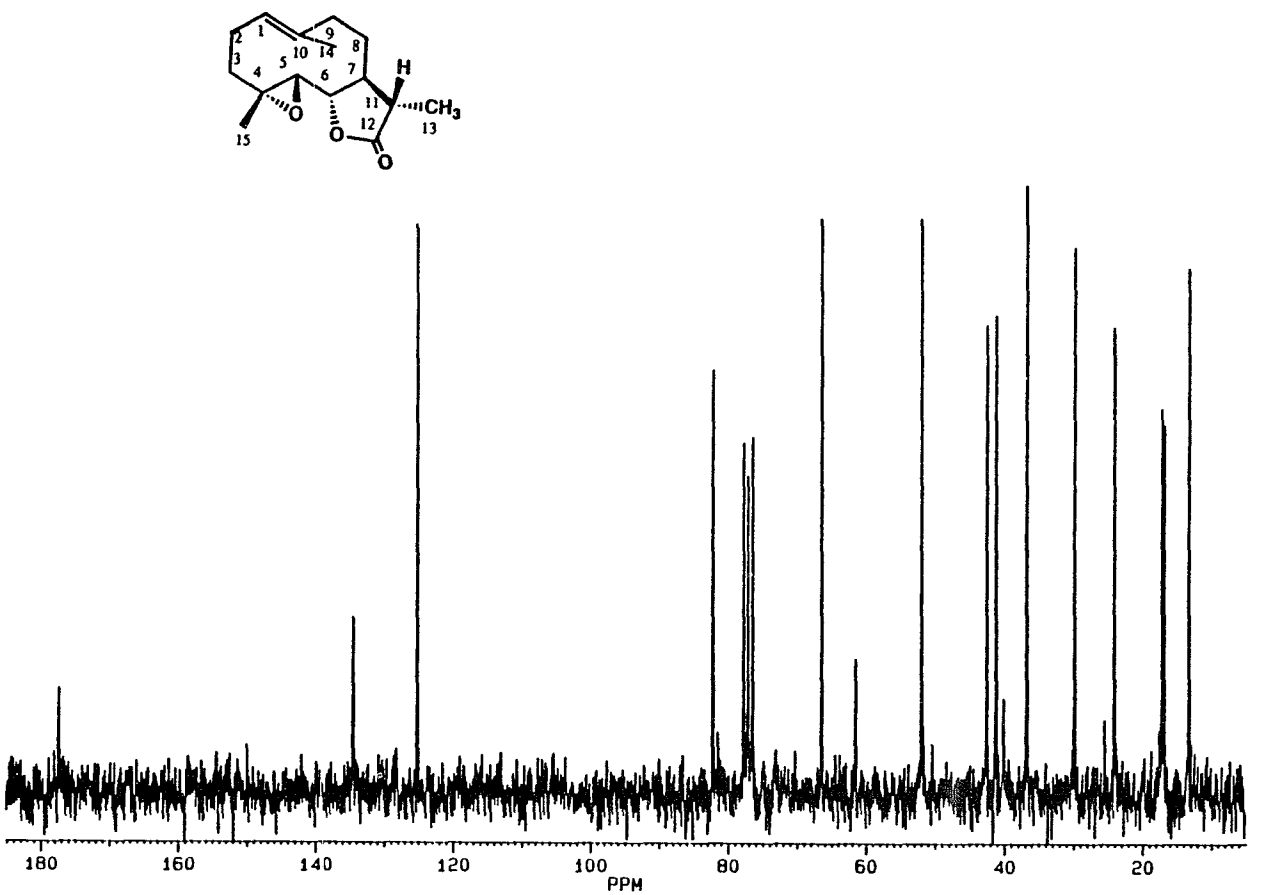


Figure 2.6. ^{13}C NMR spectrum of dihydroparthenolide **(4)** in $CDCl_3$.

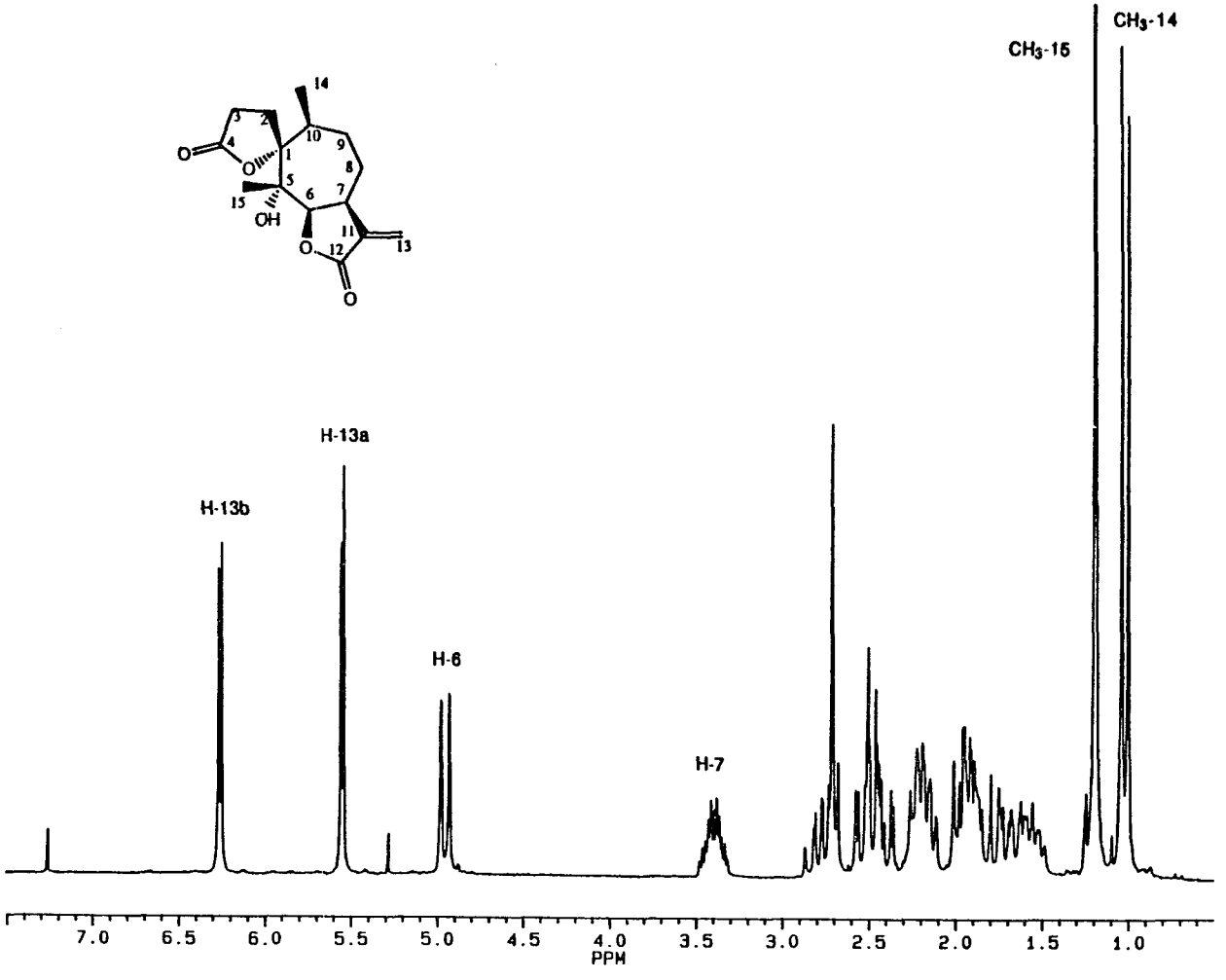


Figure 2.7. ^{1}H NMR spectrum of psilostachyin A (**5**) in $CDCl_3$.

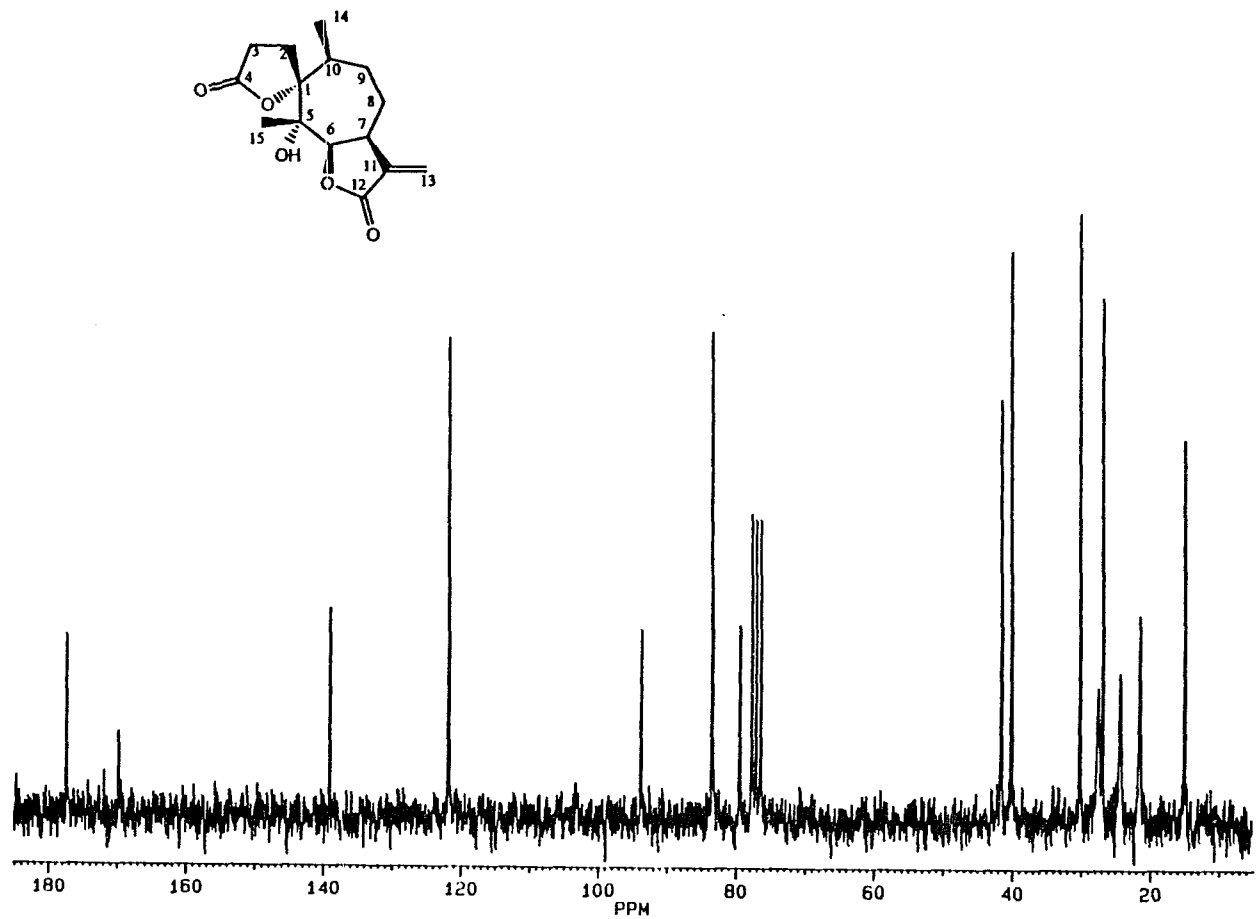


Figure 2.8. ^{13}C NMR spectrum of psilostachyin A (**5**) in $CDCl_3$.

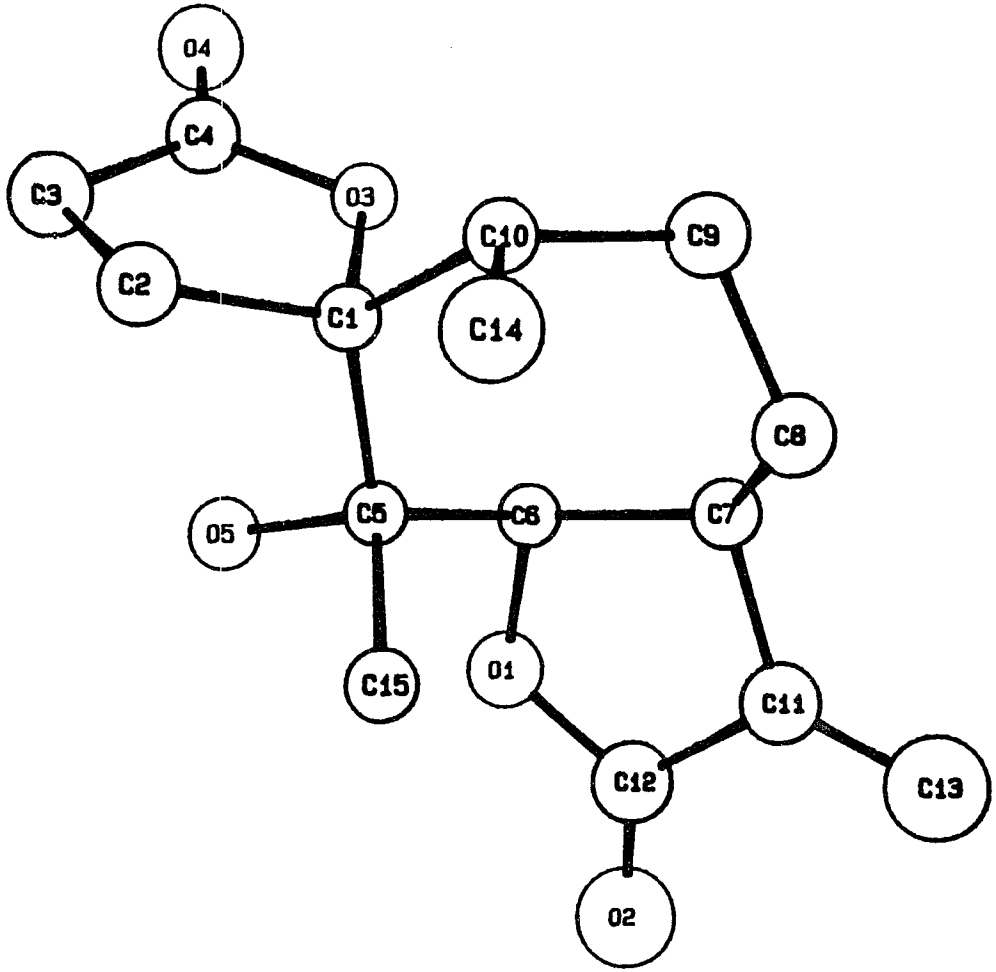


Figure 2.9. Molecular structure of psilostachyin A **(5)**.

References

1. Parodi, F.J. Dissertation. "Structure Elucidation of Natural Products From Asteraceae Using Modern NMR Techniques and Biomimetic Transformations of 11,13-Dihydroparthenolide." Louisiana State University, **1988**.
2. Wu, Yung-Fung. Thesis. "Attempts Toward A Biogenetic-Like Synthesis of Pseudoguaianolides From A 4,5-Epoxy-germacranolide, Dihydroparthenolide." Louisiana State University, **1977**, 45-6.
3. Fischer, N.H.; Weidenhamer, J.D.; Bradow, J.M. *Phytochemistry* **1989**, *28*, 2315-7.
4. Fischer, N.H.Sesquiterpene Lactones. in Methods in Plant Biochemistry. The Terpenoids. Vol. 7, Academic Press, London, 1991, in press.
5. Herz, W.; Hogenauer, G.C. *J. Org. Chem.* **1961**, *26*, 5011-3.
6. Coll, J.C.; Bowden, B.F.; *J. Nat. Prod.* **1986**, *49(5)*, 934-6.
7. Pelletier, S.W.; Chokshi, H.P.; Desai, H.K. *J. Nat. Prod.* **1986**, *49*, 892-900.
8. Wilzer, K.A.; Han, A.C.; Zambrano, I.C.; Fronczek, F.R.; Watkins, S.F. *Acta. Cryst. Crystal Structure Communications* **1988**, *C44*, 1221-3.
9. Govindachari, T.R.; Joshi, B.S.; Kamat, V.N. *Tetrahedron* **1965**, *21*, 1509-19.
10. Quick, A.; Rogers, D. *J. Chem. Soc. Perkin II* **1976**, 465-9.
11. Higo,A.; Hamman, Z.; Timmermann, B.N.; Yoshioka, H.; Lee, J.; Mabry, T.J.; Payne, W.W. *Phytochemistry* **1971**, *10*, 2241.
12. Borger-del-Castillo, J.; Manresa-Ferrero, M.T.; Rodriguez, F.; Vasques-Bueno, Luis,P.; Joseph-Nathan, P. *Org. Magn. Reson.* **1981**, *17*, 232-4.

Chapter 3. Enolate Oxidations of Sesquiterpene Lactones.

Part A. Enolate Oxidations With Oxygen.

Preparation of 11-Hydroxysesquiterpene Lactones as Models For PFK Inhibition

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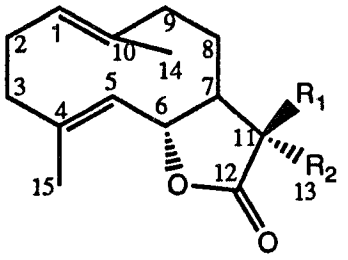
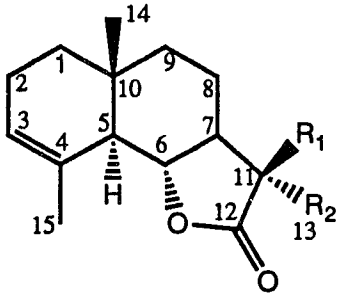
Oxidation alpha to the lactonic carbonyl group of four different skeletal types of sesquiterpene lactones has been achieved by trapping the corresponding enolates with gaseous oxygen. Both the 11α- and 11β-hydroxy-lactones are generated in combined yields ranging from 13-47%. A 14-carbon ketone product can often be isolated from the product mixture. The ketone is generated by the decomposition of a hydroperoxide intermediate.

Introduction

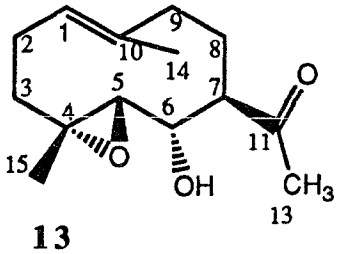
7-Hydroxy-sesquiterpene lactones are uncommon in nature, however, they show very interesting biological activities. For example, 7α-hydroxydehydrocostuslactone (**21a**) (Scheme 3.1) exhibits molluscicidal activity against *Biomphalaria glabrata* snails.[1,2] These snails are hosts in the life cycle of the blood fluke which is responsible for human schistosomiasis (or bilharzia) - a disease which affects more than 200 million people living in African, Asian, and South American countries.[3] Dehydrocostuslactone (**21b**) (Scheme 3.1) is not active against *Biomphalaria*.[1] 7α-Hydroxydehydrocostuslactone (**21a**) has also been shown to inhibit the *in vitro* activity of mammalian phosphofructokinase (PFK), and is twenty-five times more inhibitory towards PFK *in vitro* than is



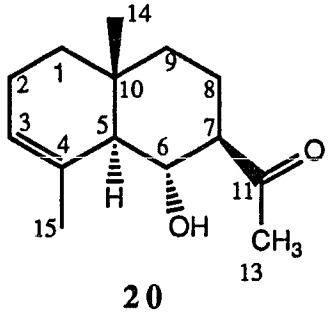
1 $R_1 = H, R_2 = CH_3$
1a $R_1, R_2 = CH_2$
2 $R_1 = CH_3, R_2 = OH$
3 $R_1 = OH, R_2 = CH_3$
4 $R_1 = H, R_2 = CH_3$, 4,5-epoxy
4a $R_1, R_2 = CH_2$, 4,5-epoxy
5 $R_1 = CH_3, R_2 = OH$, 4,5-epoxy
6 $R_1 = OH, R_2 = CH_3$, 4,5-epoxy
7 $R_1 = H, R_2 = CH_3$, 1,10-epoxy
8 $R_1 = CH_3, R_2 = OH$, 1,10-epoxy
9 $R_1 = OH, R_2 = CH_3$, 1,10-epoxy
10 $R_1 = H, R_2 = CH_3$, 1,10 and 4,5-diepoxy
11 $R_1 = CH_3, R_2 = OH$, 1,10 and 4,5-diepoxy
12 $R_1 = OH, R_2 = CH_3$, 1,10 and 4,5-diepoxy



15 $R_1 = H, R_2 = CH_3$
16 $R_1 = CH_3, R_2 = OH$
17 $R_1 = OH, R_2 = CH_3$
18 $R_1 = OOH, R_2 = CH_3$
19 $R_1 = CH_3$, 7,11-dehydro



13
14 1,10-epoxy



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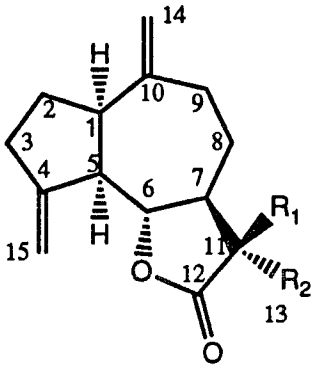
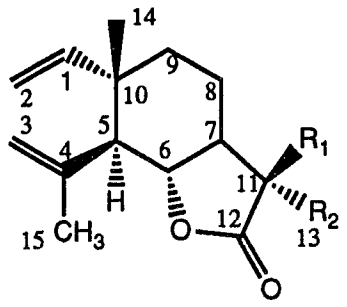
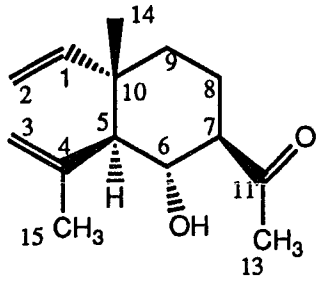
Scheme 3.1



21 $R_1 = H$, $R_2 = CH_3$
21a R_1, $R_2 = CH_2$, 7α-OH
21b R_1, $R_2 = CH_2$
22 $R_1 = CH_3$, $R_2 = OH$
23 $R_1 = OH$, $R_2 = CH_3$



24 $R_1 = H$, $R_2 = CH_3$
25 $R_1 = CH_3$, $R_2 = OH$
26 $R_1 = OH$, $R_2 = CH_3$
27 $R_1 = CH_3$, $R_2 = OOH$



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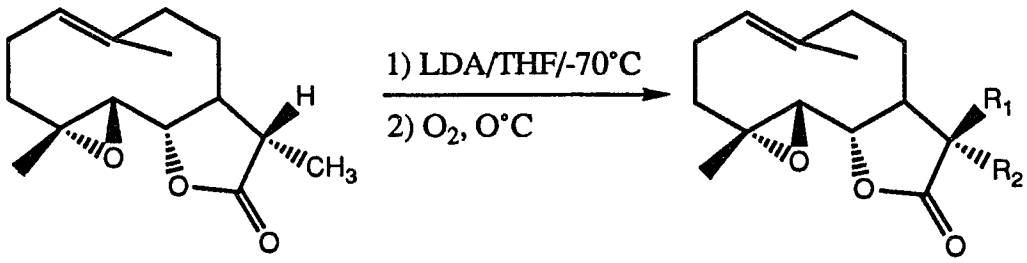
Scheme 3.1 - continued

dehydrocostuslactone (**21b**).[4] While there is no direct correlation of molluscicidal activity and PFK inhibition by sesquiterpene lactones, it is interesting to note that the most molluscicidal sesquiterpene lactone also is the most efficient inhibitor of PFK.[4]

Most biological activities of sesquiterpene lactones seem to depend on the prescence of the α-methylene-γ-lactone moiety which acts as a receptor toward biological nucleophiles like thiol groups present in various enzymes and proteins.[5,6] While the prescence of the α-methylene-γ-lactone moiety certainly enhances the inhibition of PFK, Vargas et al.[4] showed that a hydroxyl group located in proximity to the lactone functionality of sesquiterpene lactones also enhances inhibition. The 7-hydroxy-sesquiterpene lactone is probably mimicking fructose, which might initially hydrogen bond to an active site of the enzyme. We reasoned that 11-hydroxy-sesquiterpene lactones might equally well mimic sugar molecules and thus proceeded to develop a methodology to synthesize a series of 11-hydroxylated sesquiterpene lactones as models to study PFK inhibition and also molluscicidal activity.

A series of α-hydroxy-γ-lactones has been prepared from the corresponding natural sesquiterpene lactones by reacting the enolates of the lactones with oxygen[7] (Schemes 3.1 and 3.2). The reaction has been successful for four different skeletal types of sesquiterpene lactones: germacrolides, eudesmanolides, guaianolides, and elemanolides. The reaction is not stereospecific and affords both 11α- and 11β-hydroxy-derivatives. A decomposition product often resulted due to the breakdown of the hydroperoxide intermediate. The yields of the 11-hydroxylactone products ranged from 13-47%.



4

5 $R_1 = CH_3$, $R_2 = OH$

6 $R_1 = OH$, $R_2 = CH_3$

Scheme 3.2

Results and Discussion

Dihydroparthenolide (**4**) was oxidized in the following manner (Scheme 3.2). The enolate of **4** was generated at -70°C in tetrahydrofuran (THF) by deprotonation with lithium diisopropylamide (LDA) under argon atmosphere. Oxygen, dried over P_2O_5, was then bubbled through the solution for twenty minutes. The reaction was quenched by the addition of 3-4 ml of distilled water. The solution was then carefully neutralized with 5% HCl and extracted with diethyl ether. Sesquiterpene lactones (**1**), (**10**), (**15**), (**21**), and (**24**) were oxidized under similar conditions. The products were separated using silica gel column chromatography, preparative thin-layer chromatography, or HPLC.

The 11-hydroxy-derivatives were analyzed using infrared, ^{1}H NMR, and mass spectral data. The infrared spectra of these derivatives clearly showed a broad absorption signal at ~3400cm^{-1} due to the 11-hydroxyl group. The ^{1}H NMR data indicated hydroxylation by the collapse of the CH_3-13 doublet of the dihydro-lactones to a methyl singlet. The ^{1}H NMR data was also used to distinguish between the 11α- and 11β-hydroxy- derivatives. The chemical shift of the lactonic signal (H-6) for all of the 11β-hydroxy-derivatives had shifted downfield ~0.5ppm (with respect to the corresponding non-hydroxylated dihydroderivatives) due to a deshielding effect by the hydroxy group. The chemical shift of H-6 for all of the 11α-hydroxy-derivatives is approximately the same as the chemical shift of their corresponding dihydroderivatives (Table 3.1). The total yield of alcohol products from these reactions ranges from 13-47% with no apparent consistent stereoselectivity (Table 3.2).

Additional compounds (**13, 14, 20, and 28**) were in some instances isolated from the product mixture and in some cases they represented the only product

Table 3.1. Selected ^{1}H NMR data[a]

Sesquiterpene lactone	CH_3 - 13	H - 6
Dihydroparthenolide (**4**)	1.26(d)	3.80(dd)
11-α-Hydroxydihydroparthenolide (**5**)	1.31(s)	3.79(dd)
11-β-Hydroxydihydroparthenolide (**6**)	1.39(s)	4.12(dd)

[a] Chemical shifts in ppm, multiplicity in parentheses, s=singlet, d=doublet

Table 3.2. Yield (%) of Products From Enolate Oxidation of Sesquiterpene Lactones[a]

Sesquiterpene lactone	Total Yield of 11-OH-products	11α-OH	11β-OH	Decomposition Product
1	37	15	22	--
4	47	29	18	16
10	--	--	--	37
15	24	15	9	14
21	29	12	17	--
24	13	5	8	13

[a]Yields are based on recovered starting material

isolated. The infrared spectra of these compounds showed an absorption at ~ 3400cm^{-1} due to a hydroxyl group and one at ~1710cm^{-1} due to the carbonyl stretch of a ketone. The ^{1}H NMR data also showed a methyl singlet at 2.10-2.20 ppm, indicative of a methyl ketone. The ^{13}C NMR spectra of these compounds indicated the presence of only 14-carbons. Based on this data, structures **13**, **14**, **20**, and **28** were proposed.

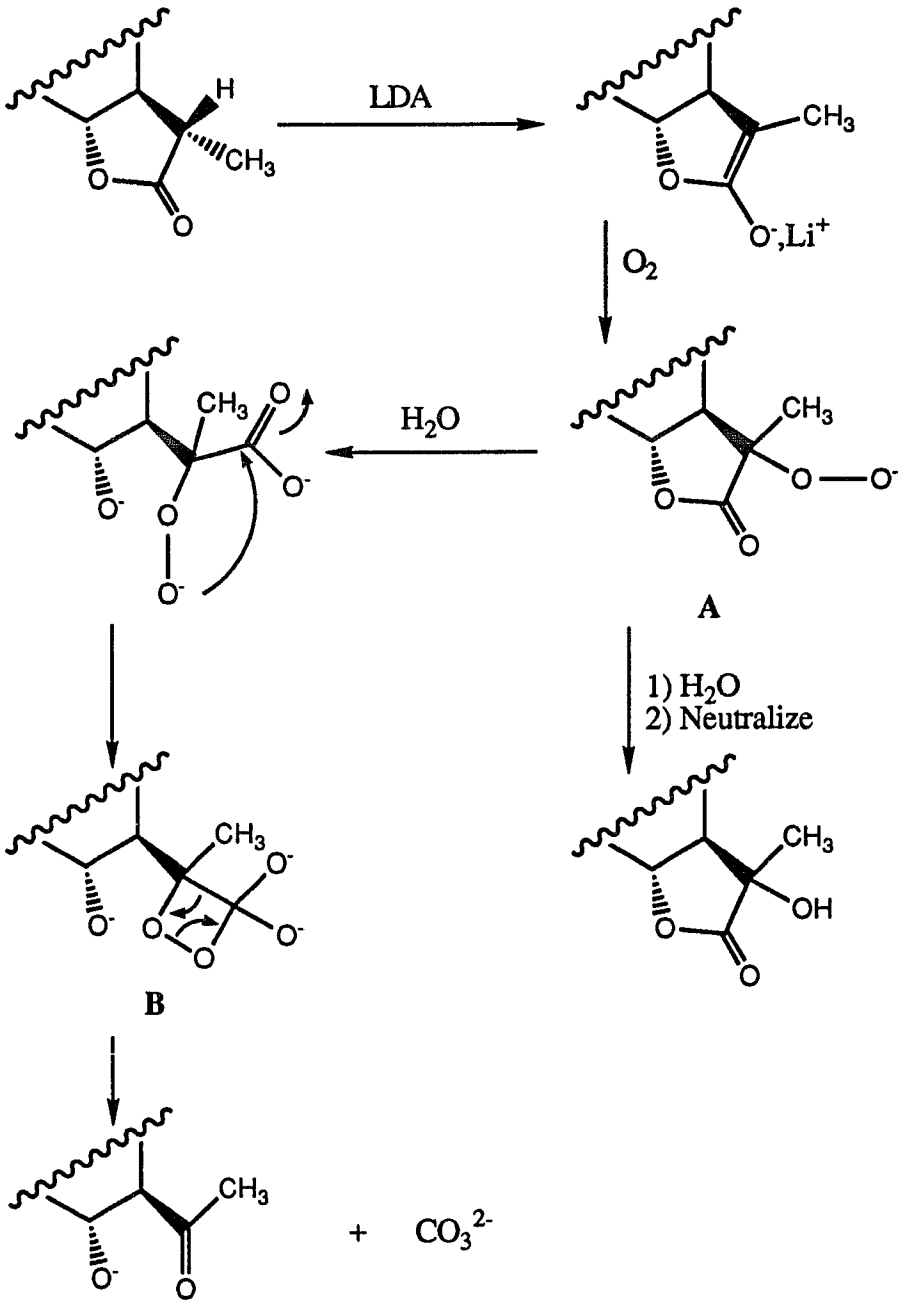
A possible mechanism for the formation of these decomposition products may involve the decarboxylation of a hydroperoxide anion intermediate (Scheme 3.3). Hydroperoxides have been reported as the major products of enolate oxidations of esters using oxygen when bases other than LDA (like tBuOK) are used.[8] The existence of a hydroperoxide intermediate was demonstrated by the isolation of **18** and **27** from their respective reaction product mixtures. The hydroperoxide intermediates (Scheme 3.3, A) are then reduced to the alcohols, probably by diisopropylamine generated in the formation of the enolate.[9] 1,2-dioxetane formation could arise following hydrolysis of the hydroperoxide anion (Scheme 3.3, B). 1,2-dioxetanes have been observed to decompose cleanly to carbonyl compounds[10] which would generate the decomposition products isolated.

Epoxidations of the 1,10-double bond of the 11-hydroxy-derivatives **2, 3, 4,** and **6** were achieved with m-chloroperoxybenzoic acid (m-CPBA) in the prescence of sodium acetate as a buffer to prevent cyclization.[11]

The ^{13}C NMR assignments for compounds **1-16, 20,** and **21** are listed in Table 3.3.

Experimental Section

^{1}H and ^{13}C NMR spectra were recorded on a Bruker-AC200 spectrometer in



Scheme 3.3

Table 3.3. ^{13}C NMR data for compounds 1-16, 20, and 21.[a]

Carbon atom	1	2	3	4[13]	5	6	7	8	9	10	11
1	127.0	127.0	127.1[b]	125.1	125.0	124.5	67.4	67.7	67.5	64.1[b]	64.3[b]
2	25.7	24.0	23.2	24.0	23.9	23.9[b]	24.5	24.6[b]	21.8	23.6	20.8
3	40.6	40.9	41.2	36.6	36.6	36.8	35.9	36.1	36.3	34.9	35.3
4	136.5	136.9	137.2	61.4	62.0	61.7	143.0	143.7	143.4	60.2[c]	60.5[c]
5	126.4	127.0	126.7[b]	66.3	66.2	66.5	123.9	123.9	124.0	63.4[b]	64.2[c]
6	80.9	79.5	81.1	82.1	80.8	82.4	80.2	78.4	79.9	81.2	80.0
7	54.2	55.9	56.4	51.9	53.2	53.7	54.9	56.5	56.9	51.2	53.3
8	28.0	26.1	25.9	29.7	24.7	24.1[b]	25.6	25.0[b]	24.6	25.0	23.8
9	39.1	39.5	39.5	41.1	41.0	41.2	39.2	39.2	39.6	39.7	39.9
10	139.6	140.6	140.7	134.4	134.5	135.1	61.1	61.3	61.4	60.3[c]	60.8[c]
11	41.7	75.3	75.5	42.4	75.5	75.3	42.1	75.2	75.3	42.3	75.3
12	178.0	179.6	177.8	177.3	178.8	176.9	178.0	178.2	177.2	176.8	178.2
13	12.8	19.2	22.0	13.2	19.0	21.7	12.7	19.0	20.6	12.6	19.2
14	15.6	16.1	16.1	16.8	16.7[b]	16.9[c]	17.3[b]	17.6[c]	17.5[b]	16.6[d]	16.9[d]
15	16.7	17.1	17.1	17.1	16.9[b]	17.0[c]	16.9[b]	17.2[c]	17.2[b]	17.1[d]	17.5[d]

a Spectra were determined in $CDCl_3$ at 200 MHz with Me_4Si as internal standard. Chemical shifts are in ppm. Assignments were made (except for **4**) by comparison with ^{13}C NMR data of similar known compounds.[17]

b-f = assignments are interchangeable

Table 3.3. ^{13}C NMR data for compounds 1-16, 20, and 21.[a]

Carbon atom	12	13	14	15	16	20	21
1	64.8[b]	125.2	64.9	23.0[e]	22.8[e]	23.3[c]	46.9
2	21.7	23.7	23.5[b]	35.7[d]	35.8[c]	34.6[b]	41.9
3	35.5	37.2	36.1	122.1	122.6	123.5	28.6
4	60.5[c]	60.2	59.3	133.0	132.7	134.4	151.6
5	63.4[b]	64.5	63.4	50.5[b]	51.0[b]	51.0	51.8
6	81.6	69.5	67.5	81.8	79.6	69.2	85.1
7	53.7	71.0	71.2	53.9[b]	56.1[b]	60.8	49.7
8	23.7	28.1	23.7[b]	23.5[e]	23.5[e]	24.5[c]	32.4[c]
9	40.3	40.1	39.1	37.6[d]	37.6[c]	38.0[b]	38.6[c]
10	60.9[c]	135.0	60.6	40.6[c]	39.1	39.5	149.8
11	74.9	209.8	210.0	39.4[c]	74.0	212.7	42.1[b]
12	176.2	---	---	179.6	180.4	---	178.5
13	19.5	29.6	30.6	12.3[f]	19.0[f]	29.4	13.1
14	16.9[d]	17.2	17.1[c]	17.2[f]	18.1[f]	16.6	111.7
15	17.4[d]	17.2	17.6[c]	22.7[f]	17.3[f]	22.9	109.0

a Spectra were determined in $CDCl_3$ at 200 MHz with Me_4Si as internal standard. Chemical shifts are in ppm. Assignments were made (except for **4**) by comparison with ^{13}C NMR data of similar known compounds.[17]

b-f = assignments are interchangeable

$CDCl_3$ using $SiMe_4$ as an internal standard. Mass spectra were recorded on a HP5985 spectrometer. Infrared spectra were recorded either on a Perkin-Elmer 257 or 1760x spectrometer in film on NaCl plates.

Chromatographic separations were made on silica gel (60-200M, J.T.Baker Chemical Co.). HPLC separations were carried out on a Milton-Roy HPLC using a RSIL-C18-10μ Semi-prep column (Alltech/Applied Science).

X-ray intensity data were collected by ω-2θ scans on an Enraf-Nonius diffractometer equipped with CuKα radiation (λ = 1.54184Å) and a graphite monochromater. Two octants of data were measured within $2° < \theta < 75°$. The structure was solved by direct methods and refined by full-matrix least squares using the Enraf-Nonius SDP. Nonhydrogen atoms were refined anisotropically, and hydrogen atoms were refined isotropically.

Dihydroparthenolide (**4**) was isolated from the dichloromethane extract of the aerial parts of *Ambrosia artimisiifolia*.[12,13] The 11,13-dehydro-derivatives of **1** and **21**, costunolide and dehydrocostuslactone, were isolated by vacuum liquid chromatography from Costus Resinoid (Pierre Chauvet, S.A.). The exocyclic methylene groups of costunolide and dehydrocostuslactone were reduced with $NaBH_4$ in methanol at O°C[14] to give **1** and **21** respectively.

Reagent grade tetrahydrofuran (THF) was freshly distilled over Li metal before use to remove any traces of water. A 1.5M solution of lithium diisopropylamide (LDA) in cyclohexane (Aldrich) was used without further purification.

Dihydrocostunolide (1) IR 1771, 1669cm^{-1}; ^1H NMR (Fig. 3.1): δ 4.67 (m,1H,C_1-H), 4.45 (d,1H,C_5-H), 4.45 (dd,1H,C_6-H), 1.56 (s,3H,C_{15}-CH_3), 1.29 (s,3H,C_{14}-CH_3), 1.11 (d,3H,C_{13}-CH_3,J=7Hz); MS *m/z* (relative intensity) 234 (M^+) (11.2), 219 (M-15$^+$) (4.8), 191 (M-43$^+$) (4.1), 178 (M-56$^+$) (3.1), 177 (M-57$^+$) (6.4).

Dihydrodehydrocostuslactone (21) IR 1770, 1638cm^{-1}; ^1H NMR (Fig.

3.18): δ 5.14 (d,1H,C_{15}-H,J=2Hz), 4.99 (d,1H,C_{15}-H,J=2Hz), 4.83 (s,br,1H,C_{14}-H), 4.73 (s,br,1H,C_{14}-H), 3.87 (d,1H,C_6-H,J=9Hz), 1.19 (d,3H,C_{13}-CH_3,J=7Hz); MS *m/z* (relative intensity) 232 (M^+) (16.9), 219 (M-13^+) (6.0), 214 (M-18^+) (1.6), 204 (M-28^+) (1.5), 192 (M-40^+) (1.5), 187 (M-45^+) (2.4).

α-Cyclodihydrocostunolide (15) was prepared via acidic transannular cyclization of **1**.[14] IR 1770cm^{-1}; ^1H NMR (Fig. 3.12): δ 5.30 (s,br,1H,C_3-H), 3.83 (dd,1H,C_6-H,J=10,11Hz), 1.75 (s,3H,C_{15}-CH_3), 1.15 (d,3H,C_{13}-CH_3,J=7Hz), 0.87 (s,3H,C_{14}-CH_3); MS *m/z* (relative intensity) 234 (M^+) (2.3), 219 (M-15^+) (51.2), 191 (M-43^+) (1.1).

Saussurea lactone (24) was prepared by thermolysis of **1**.[15] IR 1770, 1638cm^{-1}; ^1H NMR (Fig. 3.21): δ 5.80 (dd,1H,C_1-H,J=11,17Hz), 5.00 (m,4H,C_2-Ha,b,C_3-Ha,b), 4.12 (dd,1H,C_6-H,J=10,11Hz), 2.23 (d,1H,C_5-H,J=11Hz), 1.77 (s,3H,C_{15}-CH_3), 1.22 (d,3H,C_{13}-CH_3,J=7Hz), 1.08 (s,3H,C_{14}-CH_3); MS *m/z* (relative intensity) 234 (M^+) (0.5), 220 (M-14^+) (0.9), 217 (M-17^+) (0.3), 184 (M-50^+) (0.2), 162 (M-72^+) (0.2).

11-Hydroxylation of dihydrocostunolide (1). Compound **1** (325mg, 1.39mmol) dissolved in 5ml of dry THF (distilled over Li metal), was added slowly over 15 min. by syringe to a stirred solution of 1.2ml of LDA in 5ml of THF under argon at -70°C. After an additional 15 min., dry oxygen was bubbled through the solution for 20 min. at 0°C. The reaction was then quenched with 5ml of water. The solution was neutralized with 5% HCl and extracted with diethyl ether. The ether solution was dried over anhydrous Na_2SO_4, filtered, and the solvent evaporated. Column chromatography on silica gel using DCM/acetone (95:5) yielded 21mg (15%) of a colorless solid **2** and 31mg (22%) of **3**.

11α-hydroxydihydrocostunolide (2): IR 3434, 1773, 1668cm^{-1}; ^1H

NMR (Fig. 3.2): δ 4.80 (m,1H,C_1-H), 4.60 (dd,1H,C_6-H), 1.69 (s,3H,C_{15}-CH_3), 1.40 (s,3H,C_{14}-CH_3), 1.33 (s,3H,C_{13}-CH_3); MS, *m/z* (relative intensity) 250 (M^+) (1.2), 232 (M-18$^+$) (0.4), 222 (M-28$^+$) (2.6), 207 (M-43$^+$) (2.3).

11β-hydroxydihydrocostunolide (3) IR 3435, 1754cm^{-1}; ^{1}H NMR (Fig. 3.2): δ 4.94 (dd,1H,C_6-H), 4.80 (m,1H,C_1-H), 4.60 (d,1H,C_5-H,J=10Hz), 1.77 (s,3H,C_{15}-CH_3), 1.45 (s,3H,C_{13}- or C_{14}-CH_3), 1.42 (s,3H,C_{13}- or C_{14}-CH_3); MS *m/z* (relative intensity) 250 (M^+) (0.7), 222 (M-28$^+$) (2.8), 207 (M-43$^+$) (0.7).

11-Hydroxylation of dihydroparthenolide (4). Compound **4** (372mg) was reacted with LDA and oxygen as described before. Column chromatography on silica gel with hexane/EtOAc (1:1) yielded 88mg (29%) of **5**, 55mg (18%) of **6**, and 45mg (16%) of **13**. **11α-hydroxydihydroparthenolide (5)**: IR 3412,1784cm^{-1}; ^{1}H NMR (Fig. 3.3): δ 5.18 (dd,1H,C_1-H,J=10Hz), 3.79 (dd, 1H, C_6-H,J=9Hz), 2.76 (d,1H,C_5-H,J=9Hz), 1.70 (s,3H,C_{14}-CH_3), 1.31 (s,6H,C_{13}- and C_{15}-CH_3); MS *m/z* (relative intensity) 266 (M^+) (0.02), 223 (M-43$^+$) (0.07), 207 (M-59$^+$) (0.08), 43 ($C_2H_3O^+$) (100).

11β-hydroxydihydroparthenolide (6) IR 3443, 1753cm^{-1}; ^{1}H NMR (Fig. 3.3): δ 5.15 (dd,1H,C_1-H,J=2,9Hz), 4.12 (dd,1H,C_6-H,J=9Hz), 2.66 (d,1H,C_5-H,J=9Hz), 1.69 (s,3H,C_{14}-CH_3), 1.39 (s,3H,C_{13}-CH_3), 1.28 (s,3H,C_{15}-CH_3); MS *m/z* (relative intensity) 266 (M^+) (0.03), 231 (M-35$^+$) (0.04), 223 (M-43$^+$) (0.02), 207 (M-59$^+$) (0.14).

Ketone (13) IR 3438, 1761cm^{-1}; ^{1}H NMR (Fig. 3.10): δ 5.14 (dd,1H,C_1-H,J=4,7Hz), 3.56 (dd,1H,C_6-H,J=9Hz), 2.75 (d,1H,C_5-H), 2.19 (s,3H,C_{13}-CH_3), 1.65 (s,3H,C_{14}-CH_3), 1.27 (s,3H,C_{15}-CH_3); MS *m/z* (relative intensity) 238 (M^+) (0.1), 223 (M-15$^+$) (0.2), 220 (M-18$^+$) (0.7), 195 (M-43$^+$) (0.4), 177 (M-61$^+$) (6.1).

Epoxidation of 1. Compound **1** (200mg) was dissolved in 10ml of DCM and stirred at room temp. Sodium acetate (200mg) was added to the solution to buffer the epoxidation and prevent possible acid-catalyzed transannular cyclization.[11] mCPBA (220mg) was added to the suspension. After stirring at room temp. for 1hr., the solution was filtered and washed with 5% Na_2CO_3 (2x50ml) and H_2O (3x50ml). The DCM solution was dried over anhydrous Na_2SO_4, filtered, and the solvent evaporated yielding 181mg (85%) of **7**.

1,10-epoxydihydrocostunolide (7). IR 1771, 1672cm^{-1}; ^{1}H NMR (Fig. 3.4): δ 5.12 (d,1H,C_5-H,J=10Hz), 4.54 (dd,1H,C_6-H,J=10Hz), 2.61 (dd,1H,C_1-H,J=2,11Hz), 1.75 (s,3H,C_{15}-CH_3), 1.14 (d,3H,C_{13}-CH_3,J=7Hz), 1.06 (s,3H,C_{14}-CH_3); MS *m/z* (relative intensity) 250 (M^+) (0.9), 235 (M-15$^+$) (0.3), 232 (M-18$^+$) (0.3), 207 (M-43$^+$) (0.6), 193 (M-57$^+$) (1.8).

Epoxidation of 2. Compound **2** (7mg) was epoxidized as described above yielding 4mg (54%) of **1,10-epoxy-11α-hydroxydihydrocostunolide (8).** IR 3418, 1775, 1671cm^{-1}; ^{1}H NMR (Fig. 3.5): δ 5.20 (d,1H,C_5-H,J=10Hz), 4.60 (dd,1H,C_6-H,J=10Hz), 2.68 (dd,1H,C_1-H), 1.83 (s,3H,C_{15}-CH_3), 1.33 (s,3H,C_{13}-CH_3), 1.12 (s,3H,C_{14}-CH_3); MS *m/z* (relative intensity) 266 (M^+) (0.3), 221 (M-45$^+$) (0.1), 210 (M-56$^+$) (0.1), 189 (M-77$^+$) (0.7).

Epoxidation of 3. Compound **3** (9mg) was epoxidized as described above yielding 7mg of **1,10-epoxy-11β-hydroxydihydrocostunolide (9).** IR 3443, 1773, 1674cm^{-1}; ^{1}H NMR (Fig. 3.6): δ 5.15 (d,1H,C_5-H,J=10Hz), 4.97 (dd,1H,C_6-H,J=10Hz), 2.66 (dd,1H,C_1-H,J=2,11Hz), 1.80 (s,3H,C_{15}-CH_3), 1.43 (s,3H,C_{13}-CH_3), 1.14 (s,3H,C_{14}-CH_3). MS *m/z* (relative intensity) 266 (M^+) (0.1), 244 (M-22$^+$) (0.1), 222 (M-44$^+$) (0.2), 207 (M-59$^+$) (0.2), 189 (M-77$^+$) (0.3).

Epoxidation of 4. Compound **4** (150mg) was epoxidized as described above

yielding 151mg (95%) of **1,10-epoxydihydroparthenolide (10)**[16]. IR 1769 cm^{-1}; ^1H NMR (Fig. 3.7): δ 3.86 (dd,1H,C_6-H,J=9Hz), 2.77 (d,2H,C_1-H,C_5-H,J=9Hz), 1.32 (s,3H,C_{14}- or C_{15}-CH_3), 1.26 (s,3H,C_{14}- or C_{15}-CH_3), 1.19 (d,3H,C_{13}-CH_3,J=7Hz); MS *m/z* (relative intensity) 266 (M^+) (0.03), 209 (M-57$^+$) (0.06), 207 (M-59$^+$) (0.03), 205 (M-61$^+$) (0.1). The molecular structure of **10** is shown in Figure 3.26. Crystal data for **10**: $C_{15}H_{22}O_4$, MW = 266.3, orthorombic space group $P2_12_12_1$, a = 7.6414(10), b = 12.559(2), c = 14.6821(14) Å, Z = 4, Dc = 1.255gcm^{-3}, R = 0.031 for 1555 observed reflections.

Epoxidation of 5. Compound **5** (31mg) was epoxidized as described above yielding 4mg (10%) of **1,10-epoxy-11α-hydroxydihydroparthenolide (11)**. IR 3422, 1782cm^{-1}; ^1H NMR (Fig. 3.8): δ 3.86 (dd,1H,C_6-H,J=10Hz), 2.87 (d,1H,C_5-H,J=10Hz), 2.80 (dd,1H,C_1-H), 1.40 (s,3H,C_{13}-CH_3), 1.33 (s,6H,C_{14}- and C_{15}-CH_3); MS *m/z* (relative intensity) 282 (M^+) (0.03), 257 (M-25$^+$) (0.6), 219 (M-63$^+$) (0.3), 211 (M-71$^+$) (0.5), 197 (M-85$^+$) (0.9).

Epoxidation of 6. Compound **6** (25mg) was epoxidized as described above yielding 25mg (95%) of **1,10-epoxy-11β-hydroxydihydroparthenolide (12)**. IR 3391,1781cm^{-1}; ^1H NMR (Fig. 3.9): δ 4.20 (dd,1H,C_6-H,J=9Hz), 2.81 (d,1H,C_1-H), 2.80 (d,1H,C_5-H,J=9Hz), 1.46 (s, 3H,C_{13}-,C_{14}-,or C_{15}-CH_3), 1.43 (s,3H,C_{13}-,C_{14}-,or C_{15}-CH_3), 1.36 (s,3H,C_{13}-,C_{14}-,or C_{15}-CH_3); MS *m/z* (relative intensity) 282 (M^+) (0.1), 210 (M-72$^+$) (0.1), 195 (M-87$^+$) (0.1).

Hydroxylation of 10. Compound **10** (114mg) was reacted with LDA and O_2 as described before yielding 40mg of ketone **14**. IR 3449, 1711cm^{-1}; ^1H NMR (Fig. 3.11): δ 3.65 (dd,1H,C_6-H,J=9Hz), 2.86 (d,1H,C_5-H), 2.24 (s,3H,C_{13}-CH_3), 1.38 (s,3H, C_{14}- or C_{15}-CH_3), 1.30 (s,3H,C_{14}- or C_{15}-CH_3); MS *m/z* (relative intensity) 193 (M-61$^+$) (0.1), 179 (M-75$^+$) (1.4), 161 (M-93$^+$) (1.2).

Hydroxylation of 15. Compound **15** (102mg) was reacted with LDA and

O_2 as described before yielding 16mg (15%) of **16**, 10mg (9%) of **17**, 14mg (14%) of **20**, 1mg of **18**, and 1mg of **19**. **17**, **18**, and **19** were isolated by HPLC following column chromatography. **11α-Hydroxy-α-cyclodihydrocostunolide (16)** IR 3449, 1770cm^{-1}; ^{1}H NMR (Fig. 3.13): δ 5.37 (s,br,1H,C_3-H), 3.92 (dd,1H,C_6-H,J=11Hz), 2.75 (s,br,OH), 1.76 (s,3H,C_{15}-CH_3), 1.36 (s,3H,C_{13}-CH_3), 0.90 (s,3H,C_{14}-CH_3); MS *m/z* (relative intensity) 250 (M^+) (1.3), 207 (M-43$^+$) (0.4), 191 (M-59$^+$) (0.7).

11β-hydroxy-α-cyclodihydrocostunolide (17) IR 3458, 1761cm^{-1}; ^{1}H NMR (Fig. 3.14): δ 5.38 (s,br,1H,C_3-H), 4.36 (dd,1H,C_6-H,J=5Hz), 1.82 (s,3H,C_{15}-CH_3), 1.45 (s,3H,C_{13}-CH_3), 0.92 (s,3H,C_{14}-CH_3); MS *m/z* (relative intensity) 250 (M^+) (2.0), 207 (M-43$^+$) (1.2).

11α-hydroperoxy-α-cyclodihydrocostunolide (18) IR 3414, 1778cm^{-1}; ^{1}H NMR (Fig. 3.15): δ 8.73 (s,1H,OOH), 5.39 (s,br,1H,C_3-H), 3.96 (dd,1H,C_6-H,J=10Hz), 1.80 (s,3H,C_{15}-CH_3), 1.37 (s,3H,C_{13}-CH_3), 0.91 (s,3H,C_{14}-CH_3); MS *m/z* (relative intensity) 266 (M^+) (0.04), 223 (M-43$^+$) (0.5), 220 (M-46$^+$) (0.2), 216 (M-50$^+$) (0.3).

7,11-dehydro-α-cyclodihydrocostunolide (19) IR 1752, 1682cm^{-1}; ^{1}H NMR (Fig. 3.16): δ 5.43 (s,br,1H,C_3-H), 4.67 (d,1H,C_6-H,J=11Hz), 1.89 (s,3H,C_{15}-CH_3), 1.83 (s,3H,C_{13}-CH_3), 0.99 (s,3H,C_{14}-CH_3); MS *m/z* (relative intensity) 232 (M^+) (2.7), 217 (M-15$^+$) (7.3), 207 (M-25$^+$) (7.4).

Ketone (20) IR 3449, 1700cm^{-1}; ^{1}H NMR (Fig. 3.17): δ 5.35 (s,br,1H,C_3-H), 4.02 (ddd,1H,C_6-H,J=5,11Hz), 2.21 (s,3H,C_{13}-CH_3), 1.83 (s,3H,C_{15}-CH_3), 0.81 (s,3H,C_{14}-CH_3); MS *m/z* (relative intensity) 222 (M^+) (0.6), 123 (M-99$^+$) (12.7), 121 (M-101$^+$) (17.1).

Hydroxylation of 21. Compound **21** (235mg) was reacted with LDA and O_2 as described before yielding 30mg (12%) of **22** and 42mg (17%) of **23**. **11α-**

Hydroxydihydrodehydrocostuslactone (22) IR 3467, 1770, 1638cm^{-1}; ^{1}H NMR (Fig. 3.19): δ 5.16 (s,1H,C_{15}-H), 5.05 (s,1H,C_{15}-H), 4.87 (s,1H,C_{14}-H), 4.77 (s,3H,C_{14}-H), 3.87 (dd,1H,C_6-H,J=9Hz), 1.30 (s,3H,C_{13}-CH_3); MS *m/z* (relative intensity) 248 (M^+) (1.7), 220 (M-28$^+$) (1.5), 202 (M-46$^+$) (0.3), 192 (M-56$^+$) (0.2).

11β-hydroxydihydrodehydrocostuslactone (23) IR 3423, 1761, 1630cm^{-1}; ^{1}H NMR (Fig. 3.20): δ 5.20 (s,1H,C_{15}-H), 5.05 (s,1H,C_{15}-H), 4.88 (s,1H,C_{14}-H), 4.80 (s,1H,C_{14}-H), 4.20 (dd,1H,C_6-H,J=9Hz), 1.43 (s,3H,C_{13}-CH_3); MS *m/z* (relative intensity) 248 (M^+) (11.0), 204 (M-44$^+$) (2.5), 191 (M-57$^+$) (2.3), 189 (M-59$^+$) (2.3).

Hydroxylation of 24. Compound **24** (93mg) was reacted with LDA and O_2 as described before yielding 4mg (5%) of **25**, 6mg (8%) of **26**, 9mg (13%) of **28**, and less than 1mg of **27**. **11α-hydroxysaussurea lactone (25)** IR 3440, 1778, 1638cm^{-1}; ^{1}H NMR (Fig. 3.22): δ 5.79 (dd,1H,C_1-H,J=11,17Hz), 5.04 (m,4H,C_2-Ha,b,C_3-Ha,b), 4.14 (dd,1H,C_6-H,J=11Hz), 2.27 (d,1H,C_5-H,J=9Hz), 1.79 (s,3H,C_{15}-CH_3), 1.38 (s,3H,C_{13}-CH_3), 1.08 (s,3H,C_{14}-CH_3); MS *m/z* (relative intensity) 250 (M^+) (0.2), 223 (M-28$^+$) (1.7), 207 (M-43$^+$) (1.3), 189 (M-61$^+$) (1.0).

11β-hydroxysaussurea lactone (26) IR 3449, 1752, 1638cm^{-1}; ^{1}H NMR (Fig. 3.23): δ 5.80 (dd,1H,C_1-H,J=11,17Hz), 5.00 (m,4H,C_2-Ha,b,C_3-Ha,b), 4.60 (dd,1H,C_6-H,J=10,11Hz), 2.20 (d,1H,C_5-H,J=12Hz), 1.79 (s,3H,C_{15}-CH_3), 1.46 (s,3H,C_{13}-CH_3), 1.10 (s,3H,C_{14}-CH_3); MS *m/z* (relative intensity) 250 (M^+) (0.3), 204 (M-46$^+$) (0.4), 121 (M-129$^+$) (3.2).

11α-hydroperoxysaussurea lactone (27) IR 3353, 1770, 1638cm^{-1}; ^{1}H NMR (Fig. 3.24): δ 8.68 (s,1H,OOH), 5.80 (dd,1H,C_1-H,J=11,17Hz), 5.00 (m,4H,C_2-Ha,b,C_3-Ha,b), 4.17 (dd,1H,C_6-H,J=11Hz), 2.32 (d,1H,C_5-

H,J=11Hz), 1.78 (s,3H,C_{15}-CH_3), 1.39 (s,3H,C_{13}-CH_3), 1.08 (s,3H,C_{14}-CH_3); MS *m/z* (relative intensity) 266 (M^+) (0.5), 216 (M-50^+) (0.5), 166 M-100^+) (0.9).

Ketone (28) IR 3466, 1708, 1638cm-^{1}; ^{1}H NMR (Fig. 3.25): δ 5.76 (dd,1H,C_1-H,J=11,17Hz), 4.90 (m,4H,C_2-Ha,b,C_3-Ha,b), 4.10 (dd,1H,C_6-H,J=11Hz), 2.25 (s, 3H,C_{13}-CH_3), 1.78 (s,3H,C_{15}-CH_3), 1.04 (s,3H,C_{14}-CH_3); MS *m/z* (relative intensity) 222 (M^+) (2.0), 204 (M-18^+) (1.5), 189 (M-33^+) (1.0), 161 (M-61^+) (3.6).

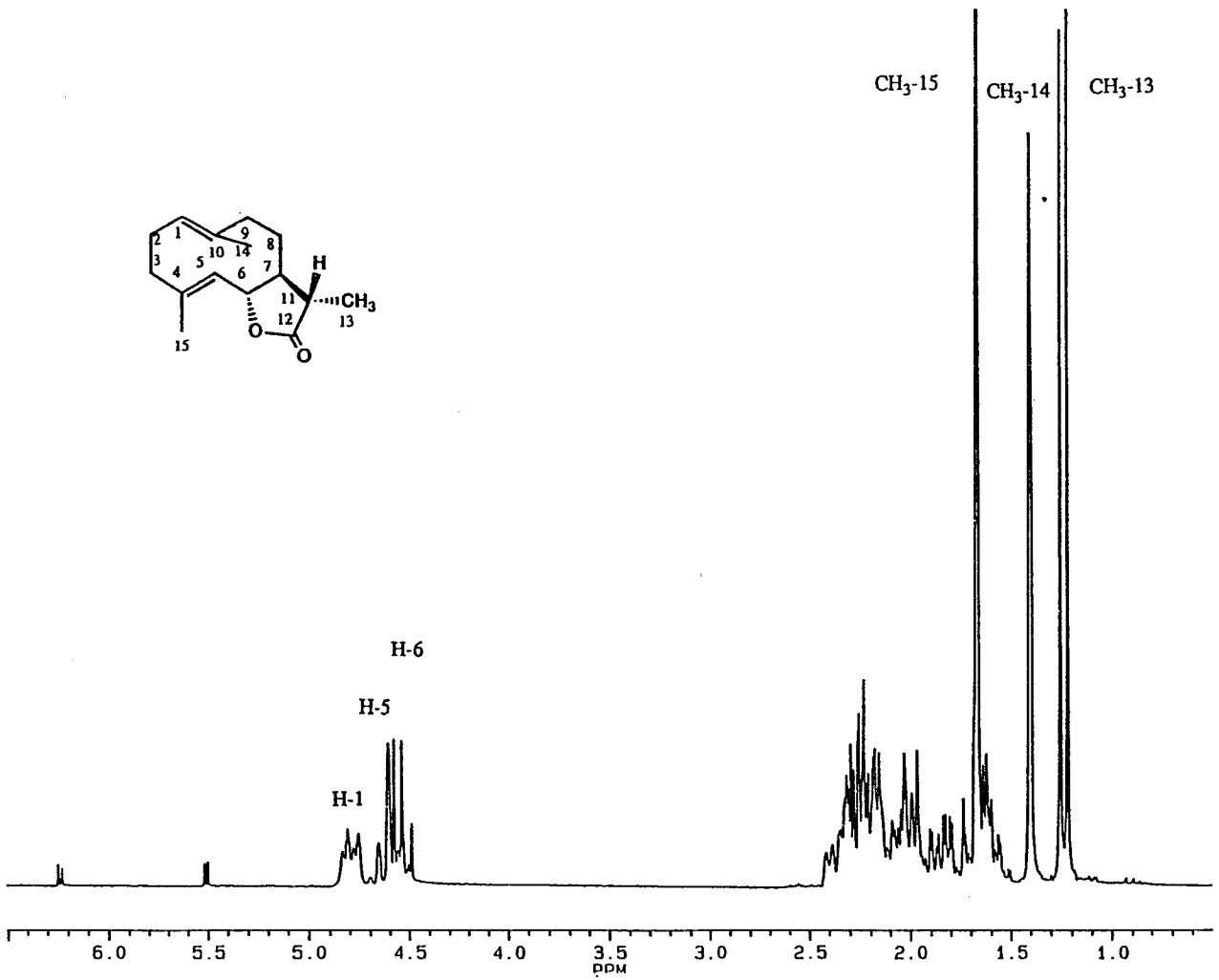


Figure 3.1. ^{1}H NMR spectrum of compound **1** in $CDCl_3$.

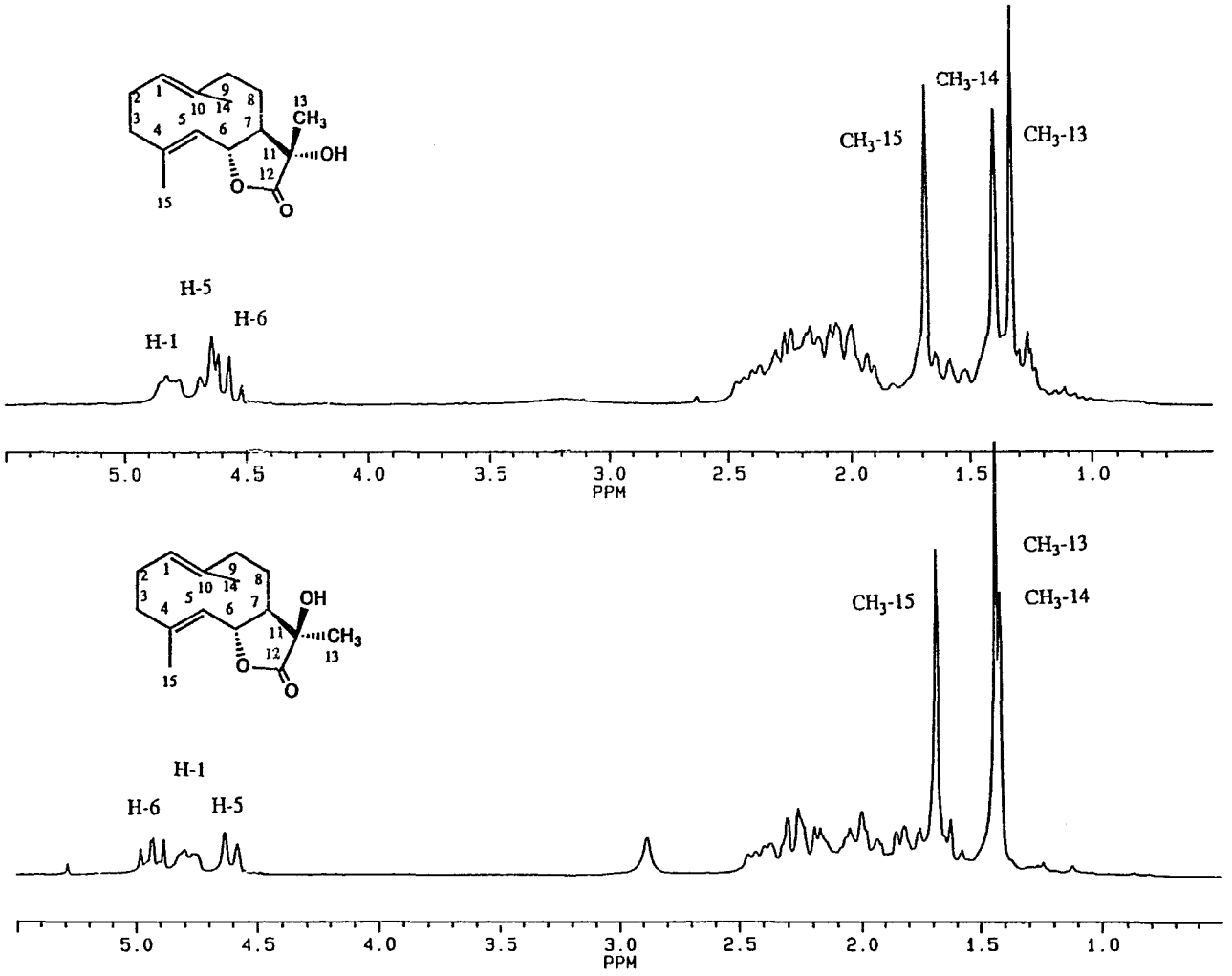


Figure 3.2. ^{1}H NMR spectra of compounds **2** and **3** in $CDCl_3$.

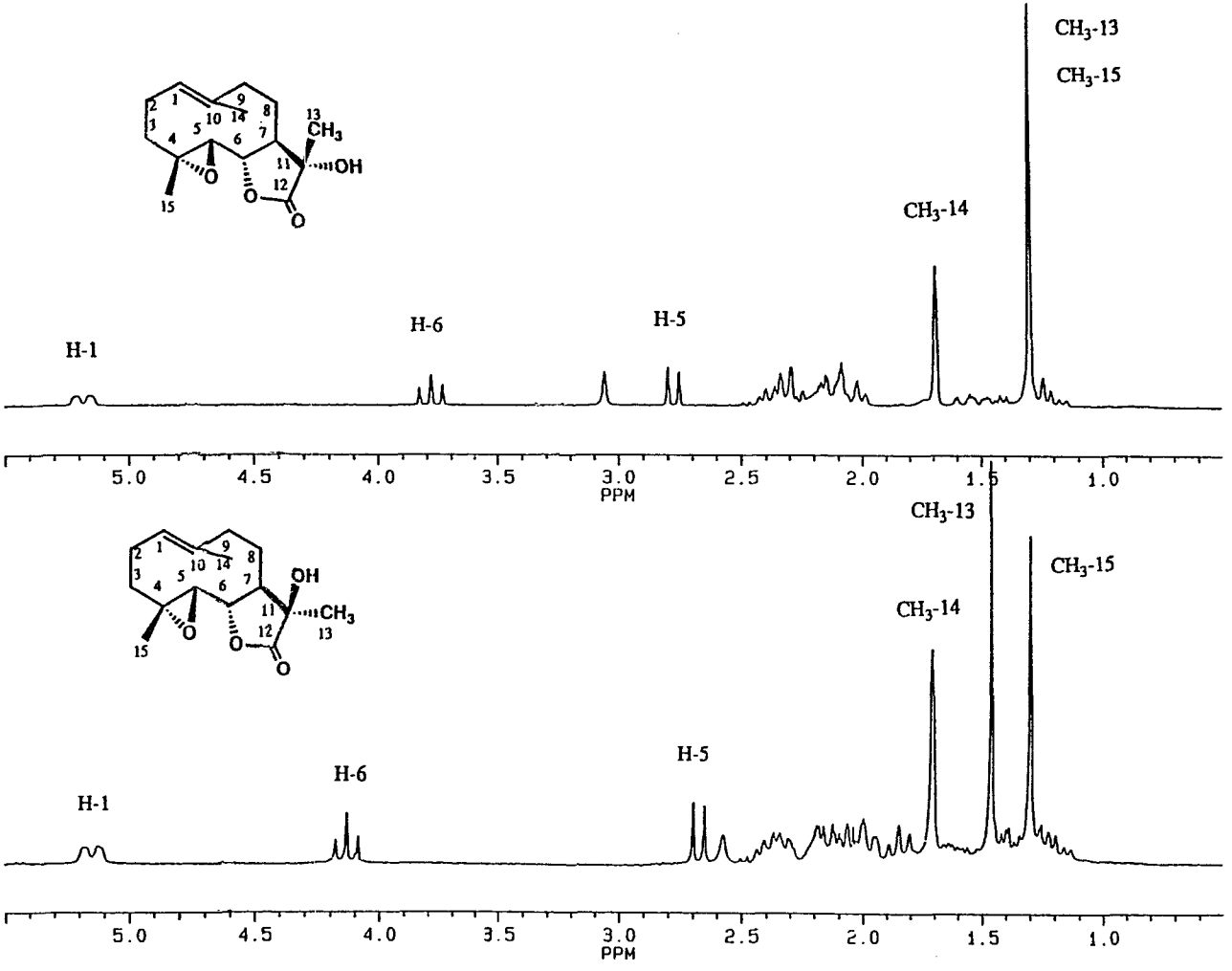


Figure 3.3. ^{1}H NMR spectra of compounds **5** and **6** in $CDCl_3$.

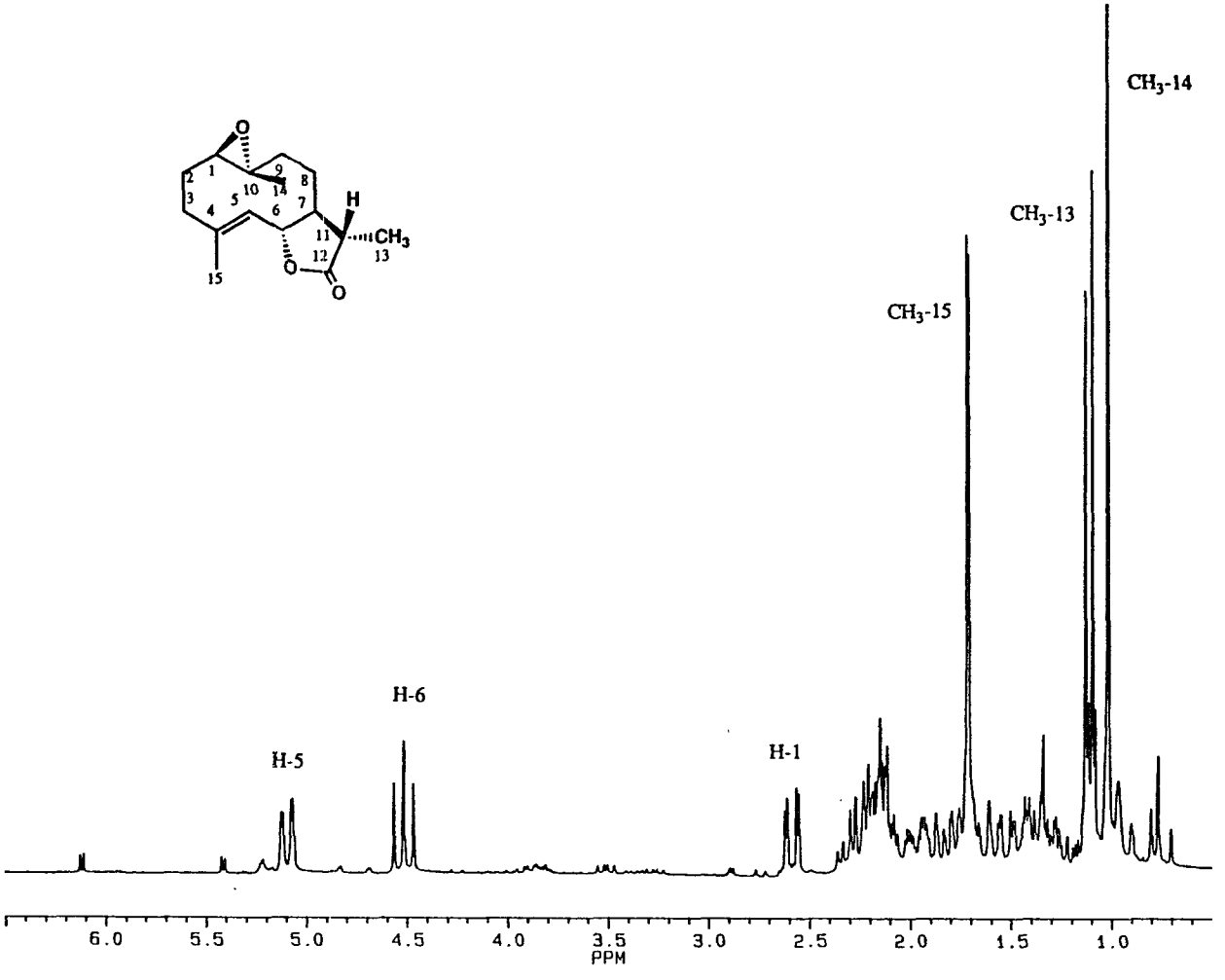


Figure 3.4. ^{1}H NMR spectrum of compound **7** in $CDCl_3$.

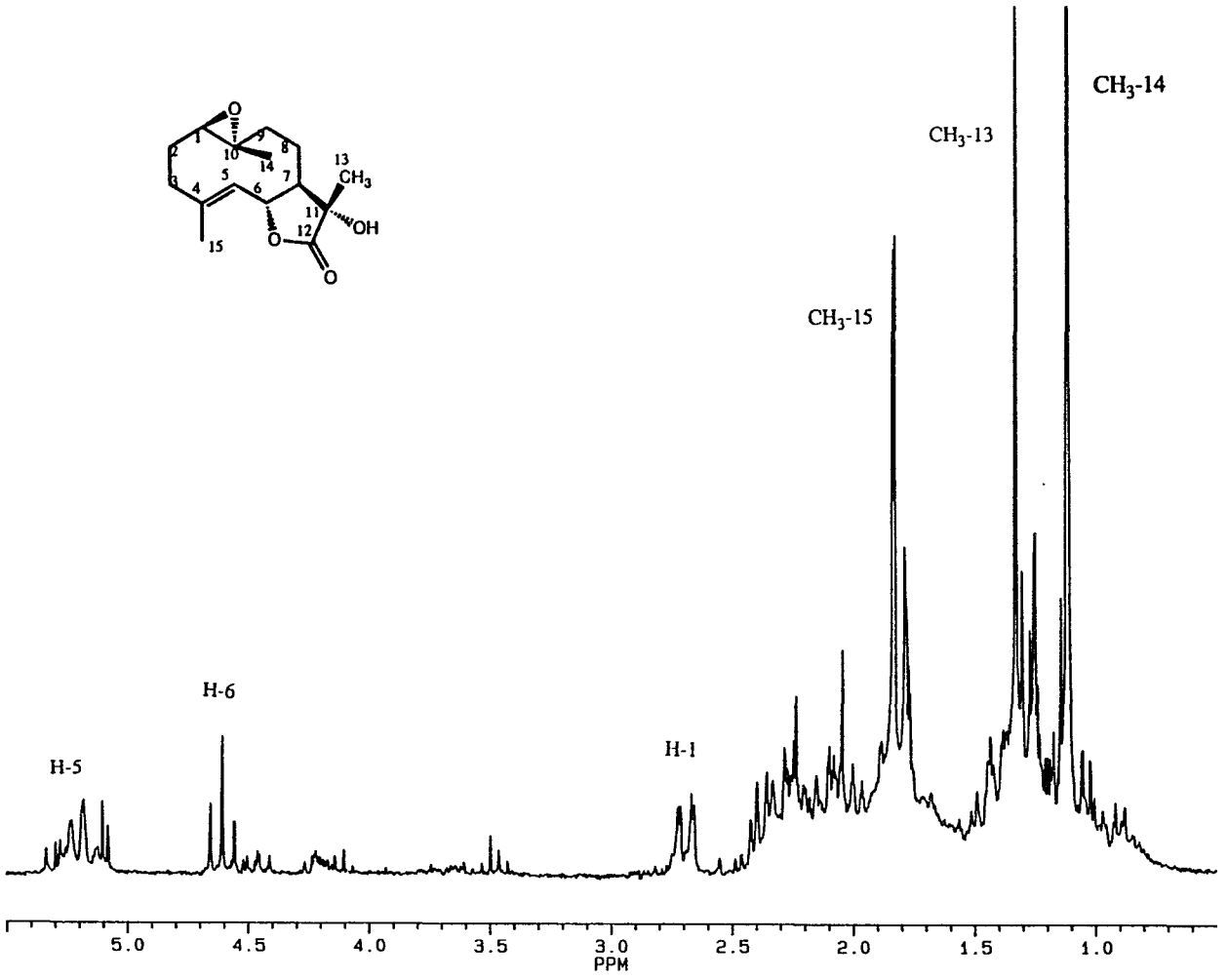


Figure 3.5. ^{1}H NMR spectrum of compound **8** in $CDCl_3$.

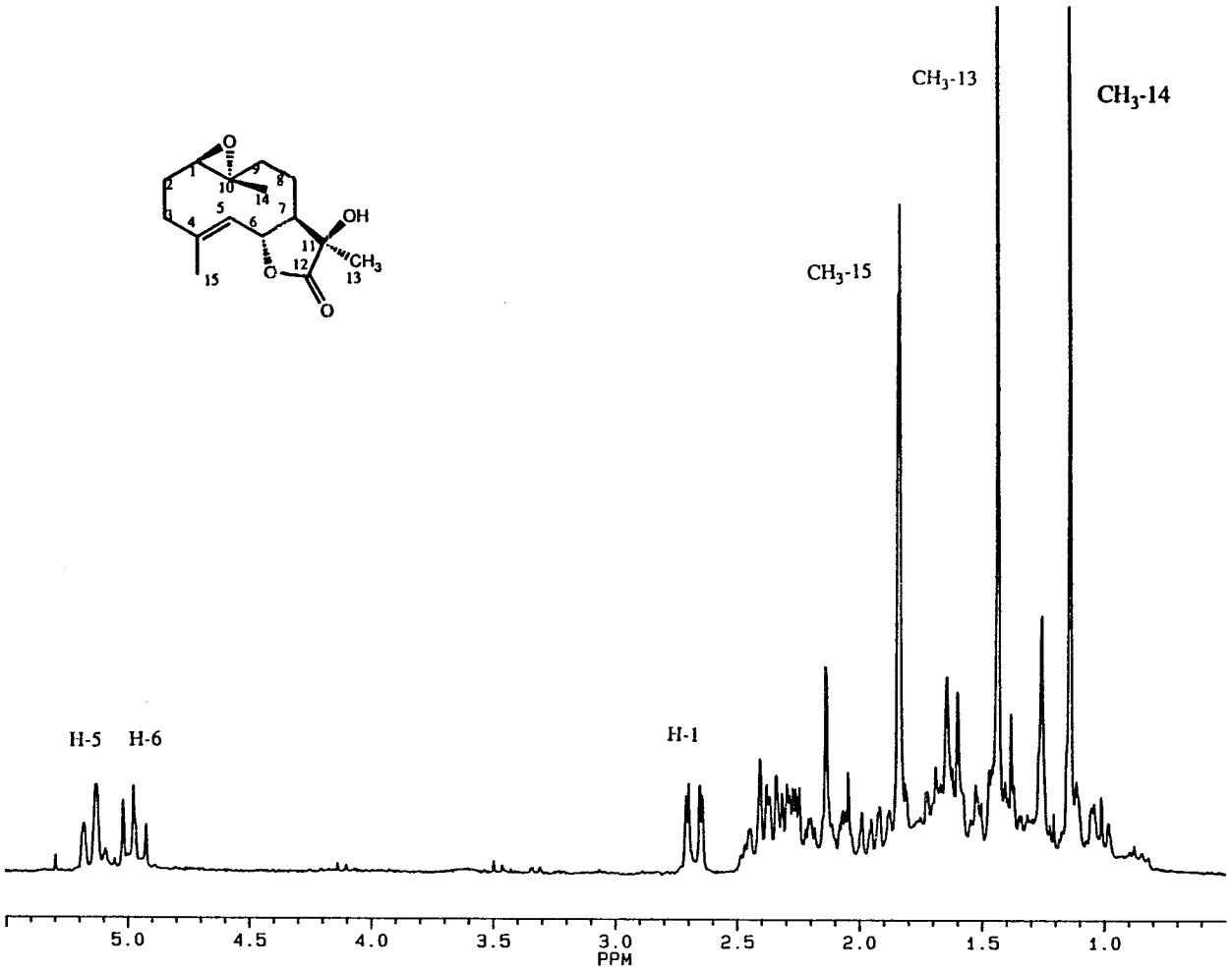


Figure 3.6. ^{1}H NMR spectrum of compound **9** in $CDCl_3$.

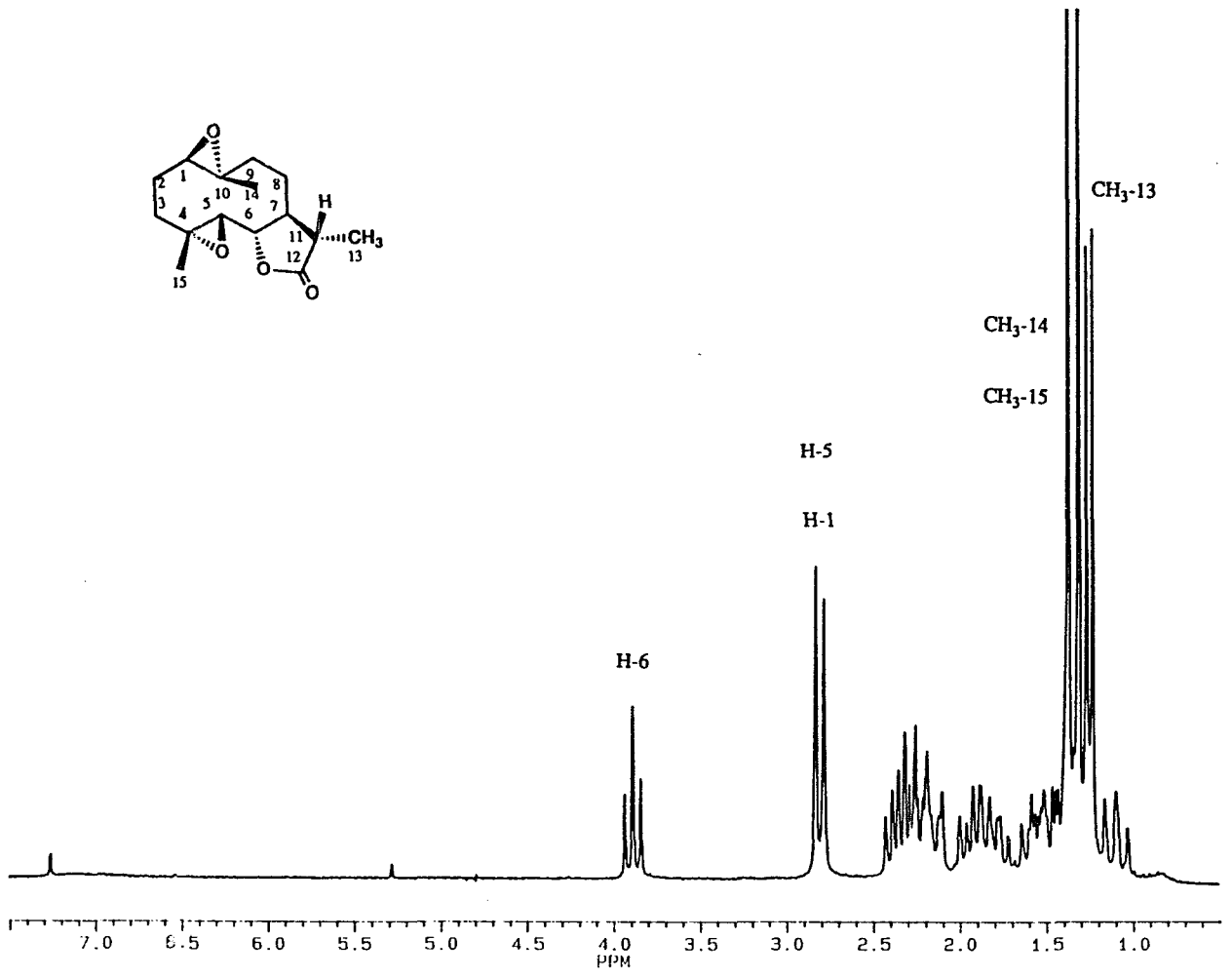


Figure 3.7. ^{1}H NMR spectrum of compound **10** in $CDCl_3$.

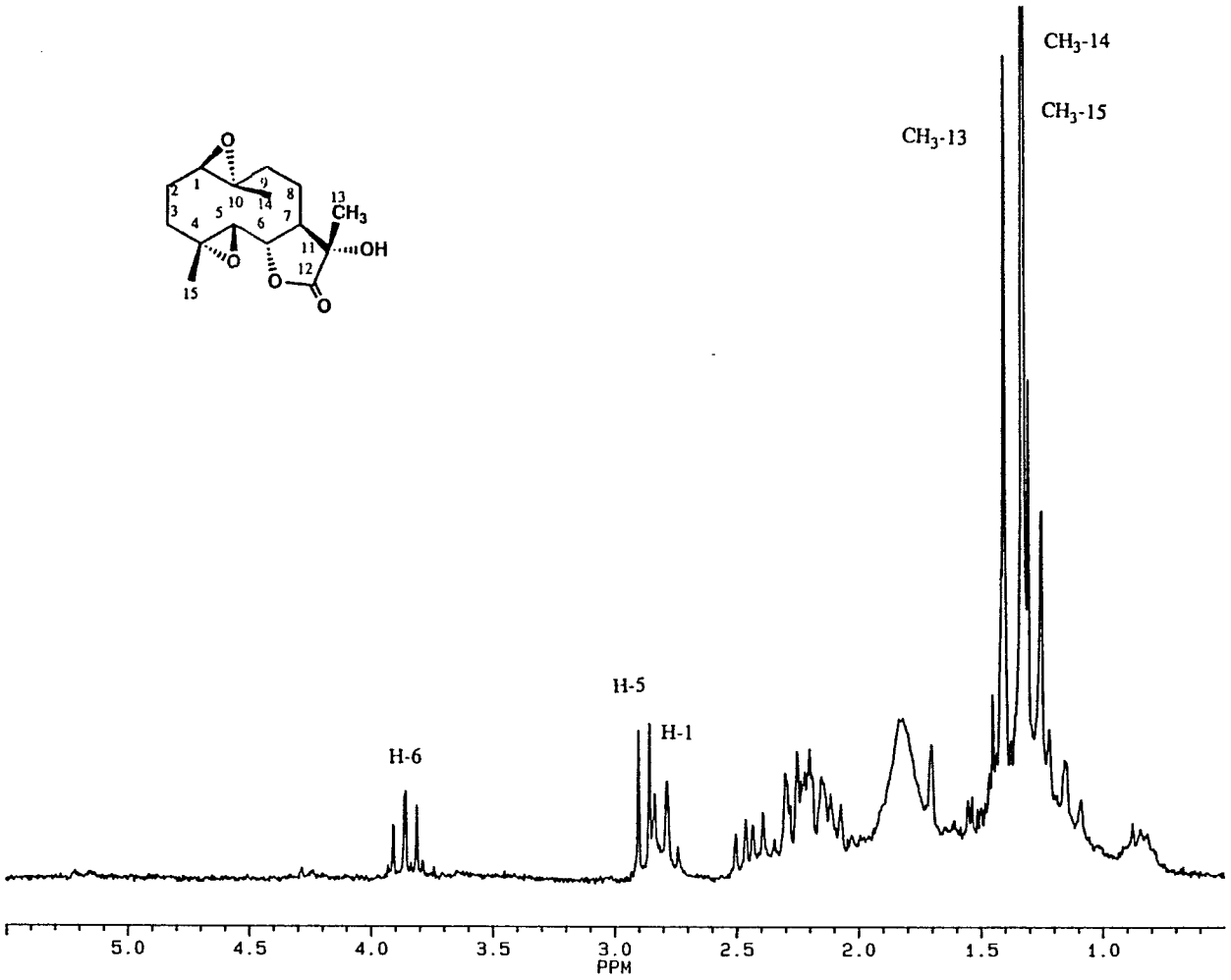


Figure 3.8. ^{1}H NMR spectrum of compound **11** in $CDCl_3$.

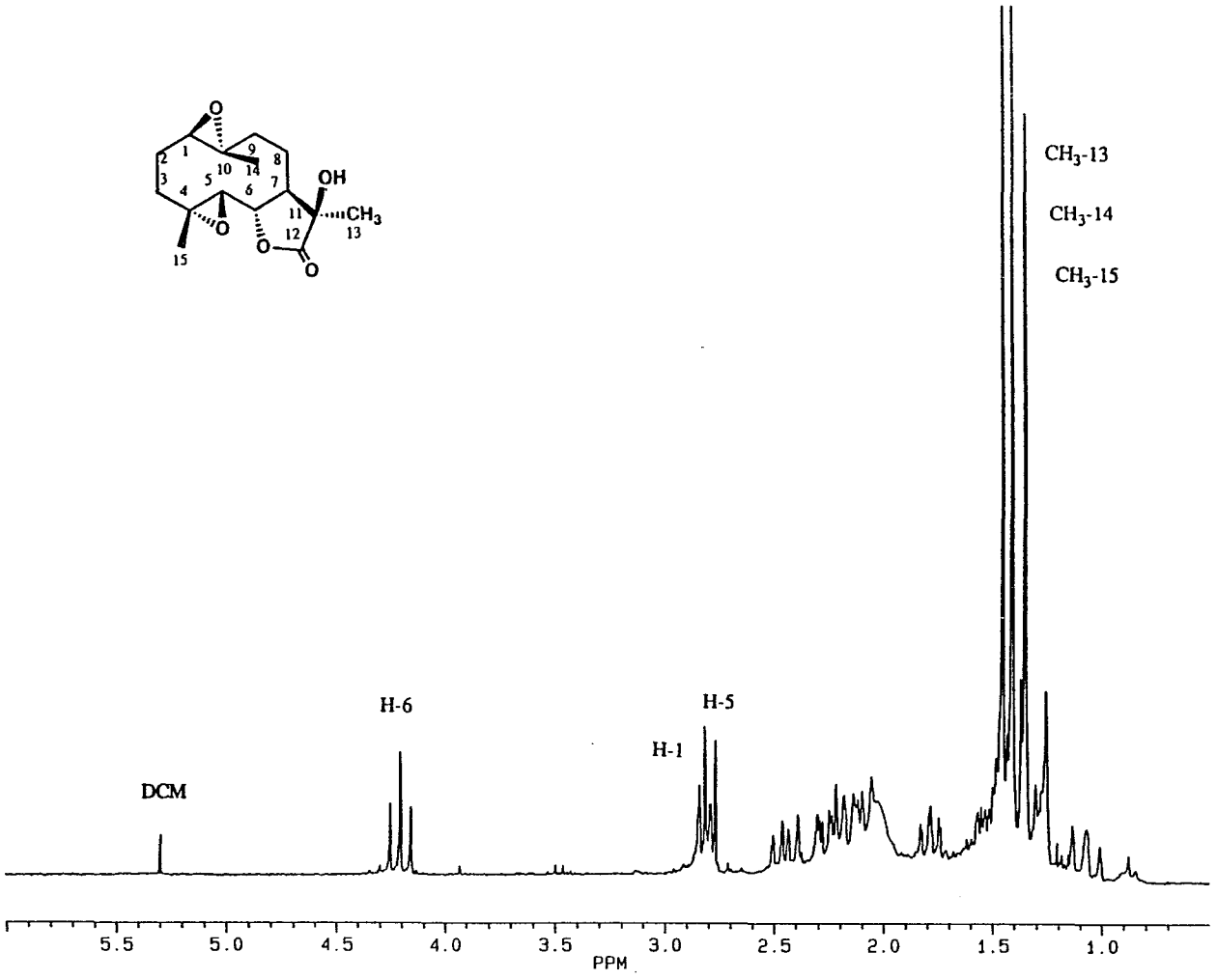


Figure 3.9. ^{1}H NMR spectrum of compound **12** in $CDCl_3$.

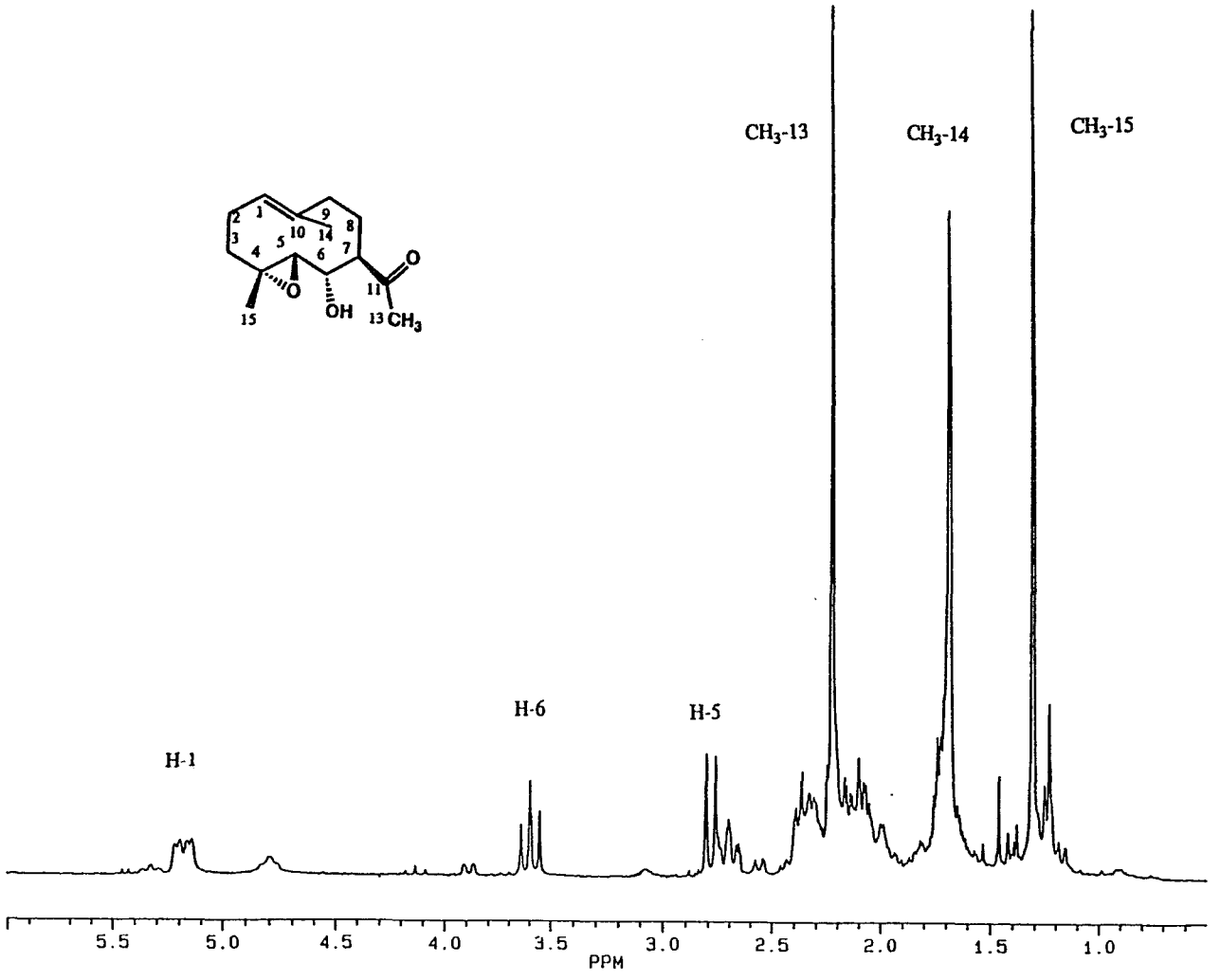


Figure 3.10. ^{1}H NMR spectrum of compound **13** in $CDCl_3$.

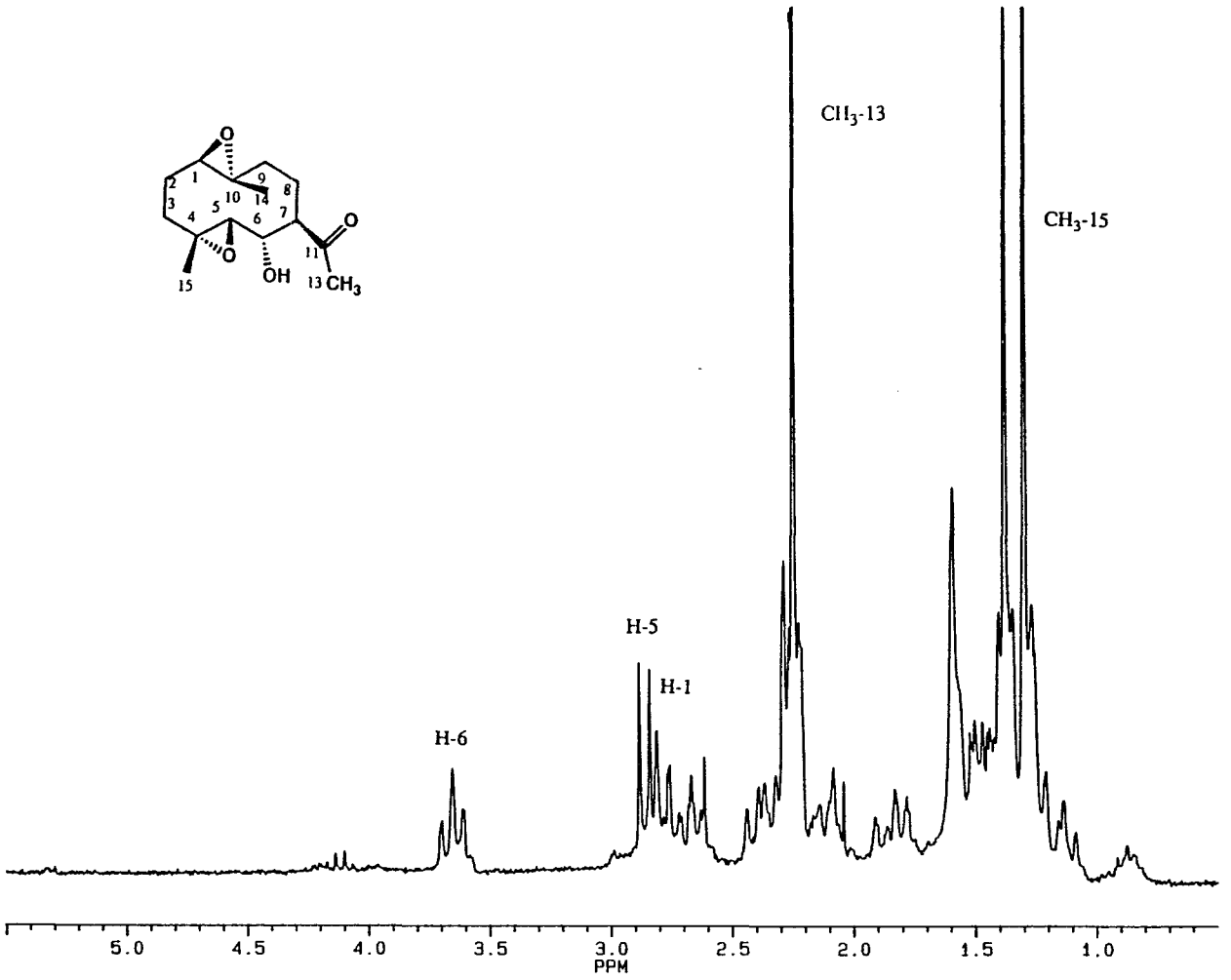


Figure 3.11. ^{1}H NMR spectrum of compound **14** in $CDCl_3$.

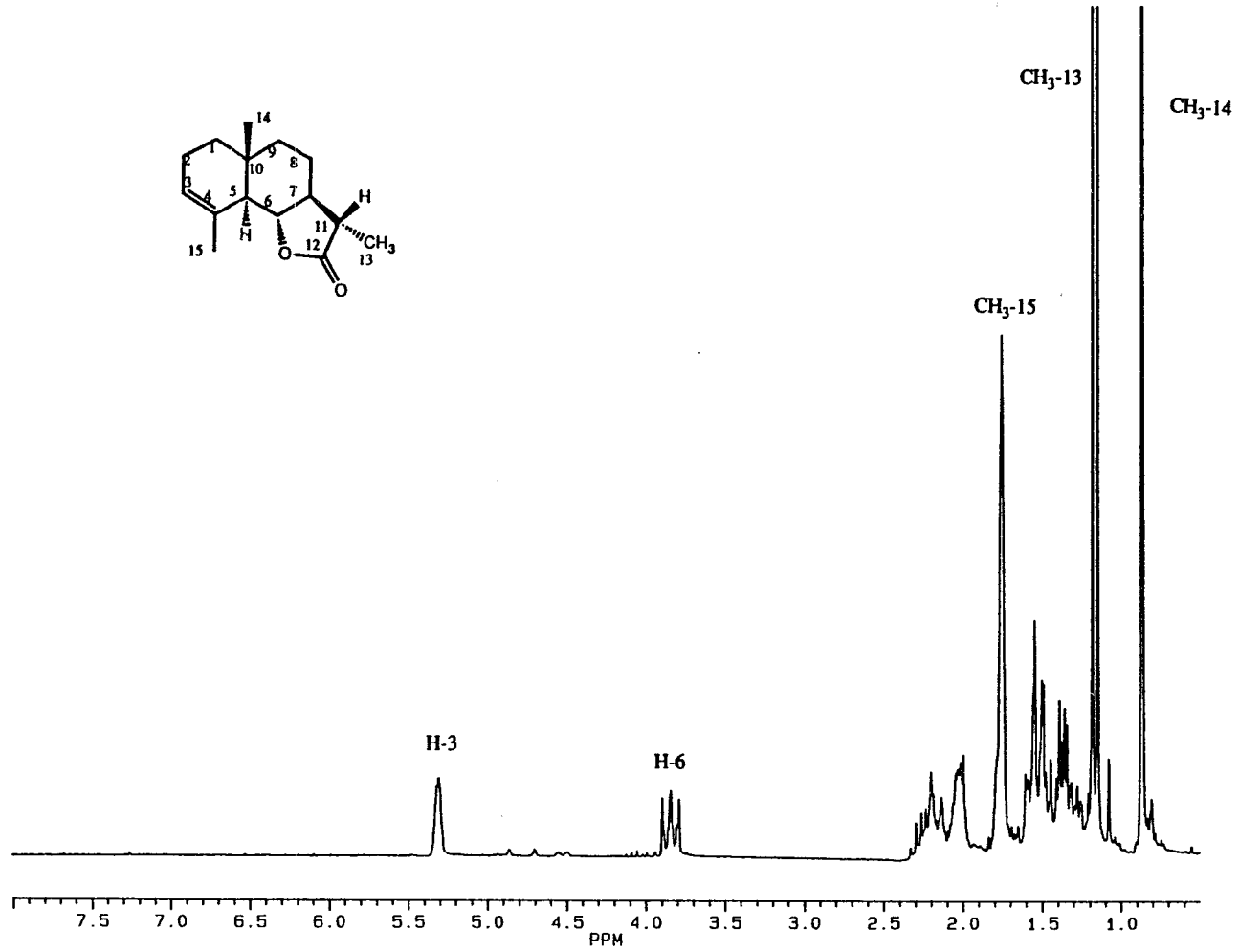


Figure 3.12. ^{1}H NMR spectrum of compound **15** in $CDCl_3$.

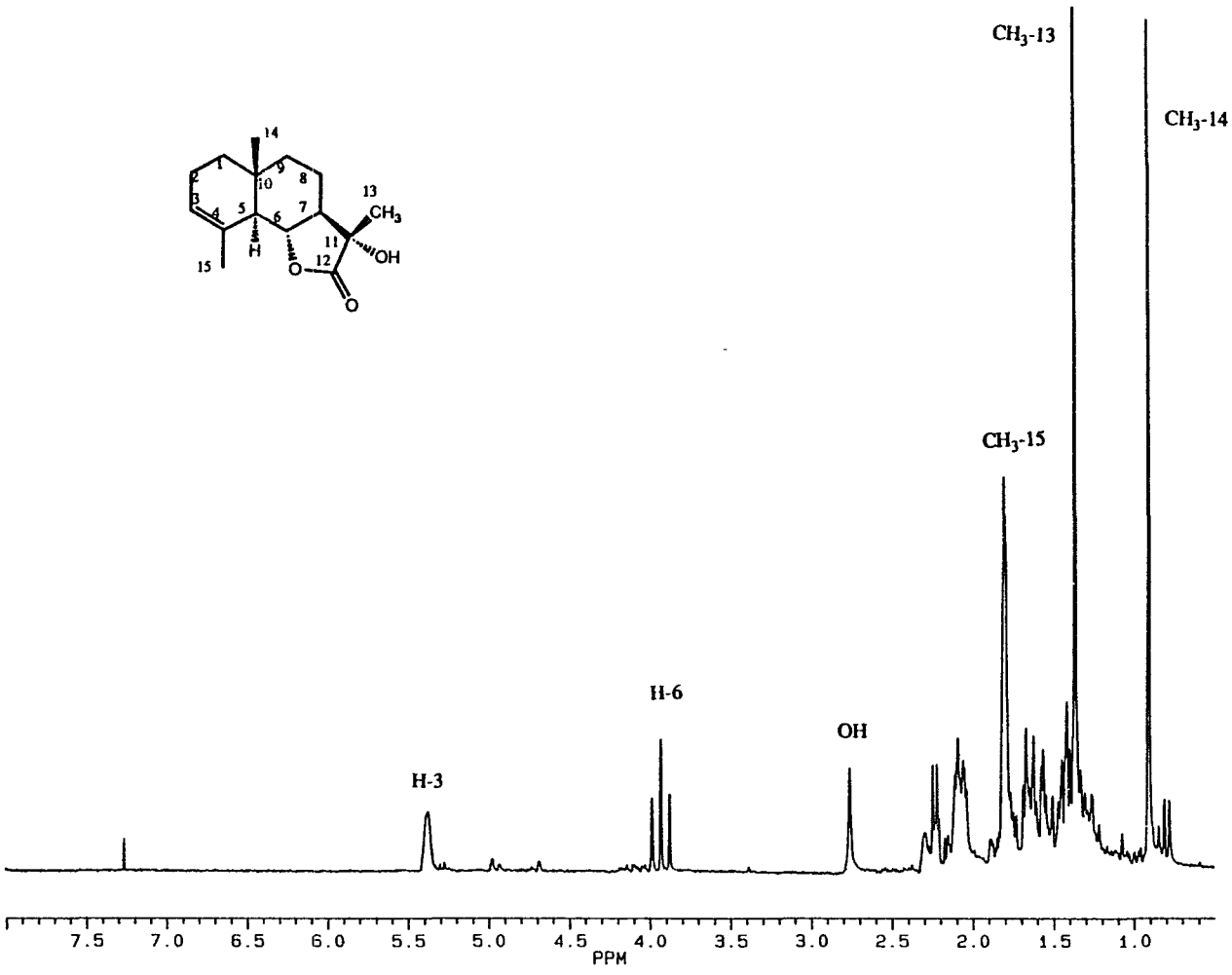


Figure 3.13. ^{1}H NMR spectrum of compound **16** in $CDCl_3$.

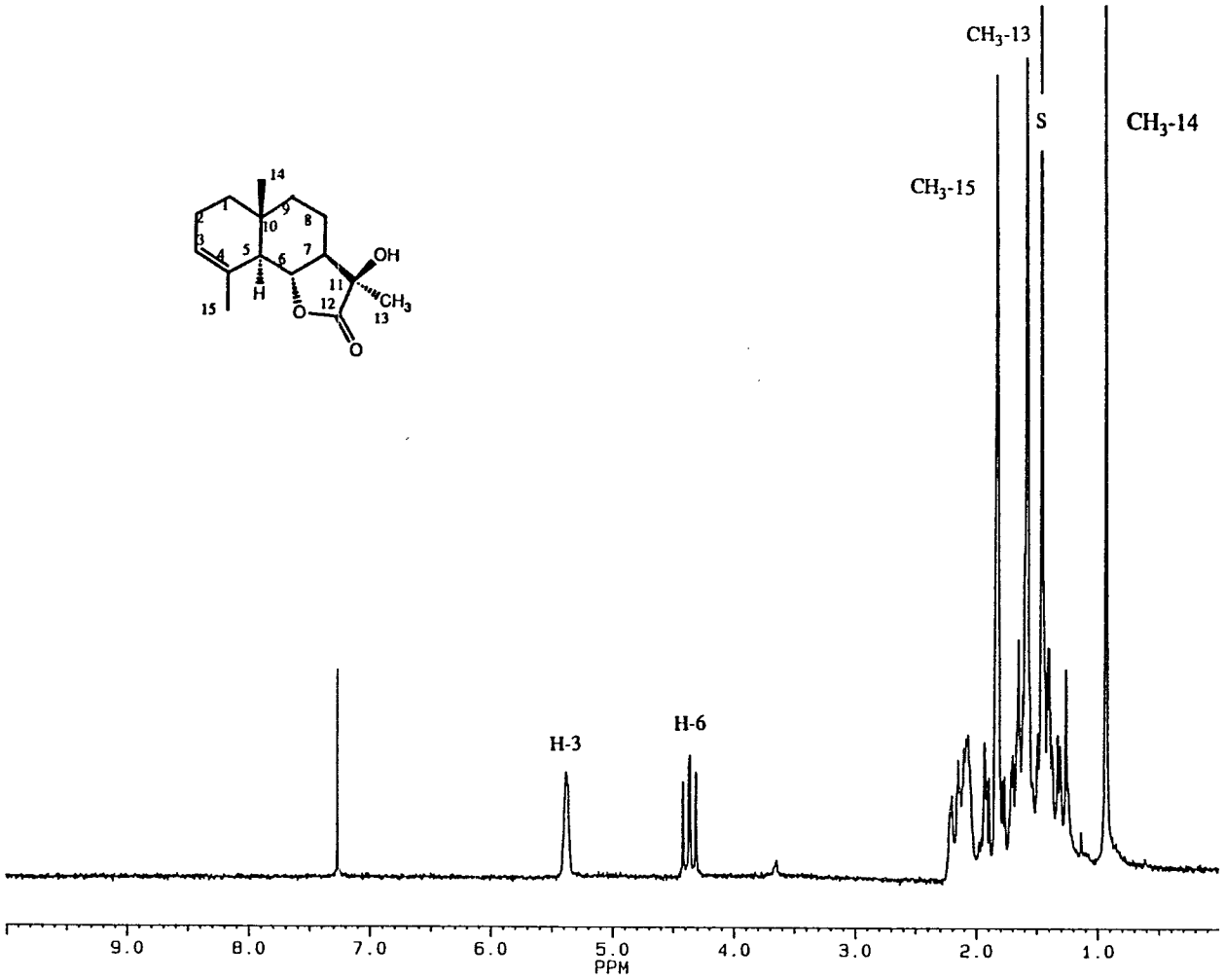


Figure 3.14. ^{1}H NMR spectrum of compound **17** in $CDCl_3$.

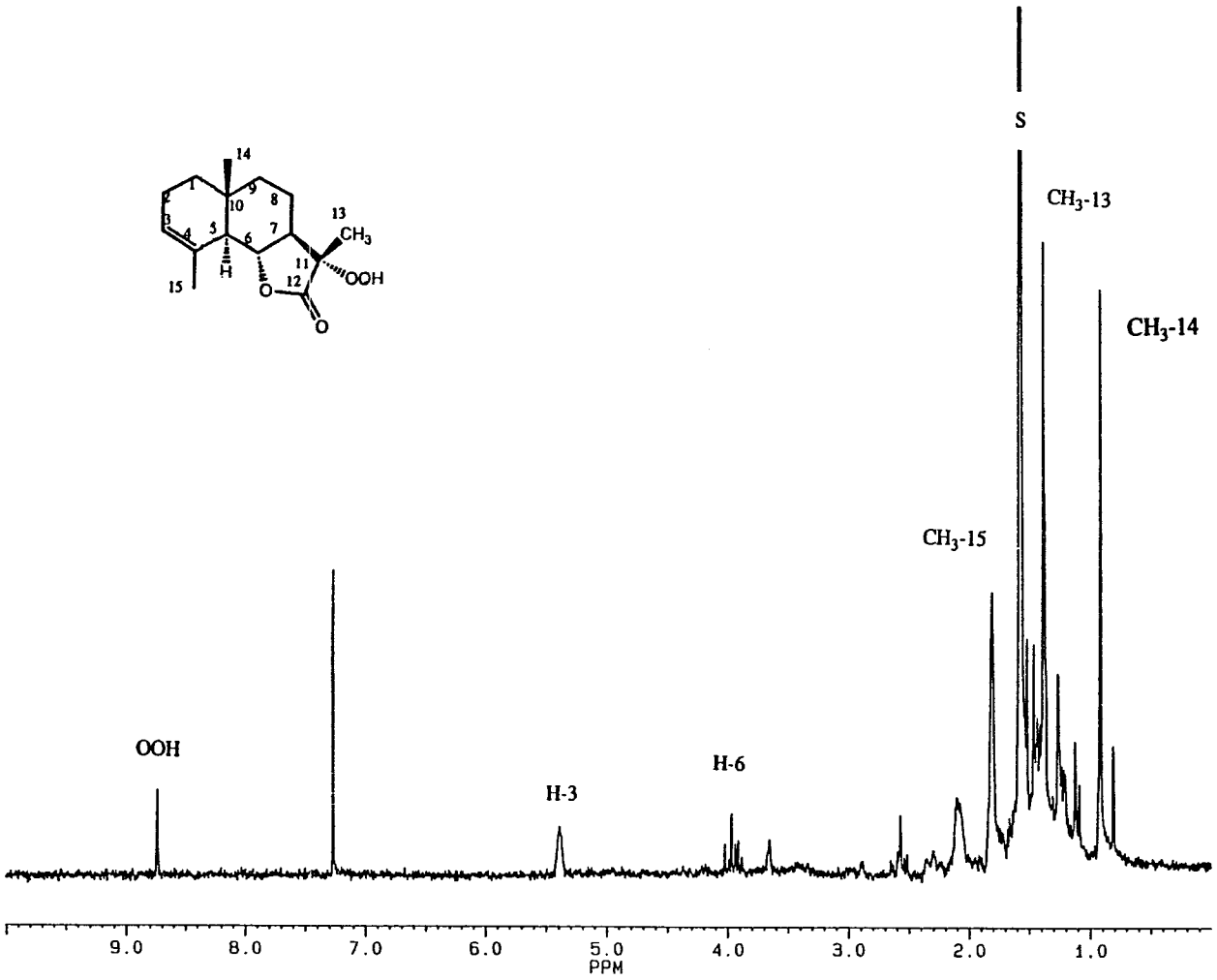


Figure 3.15. ^{1}H NMR spectrum of compound **18** in $CDCl_3$.

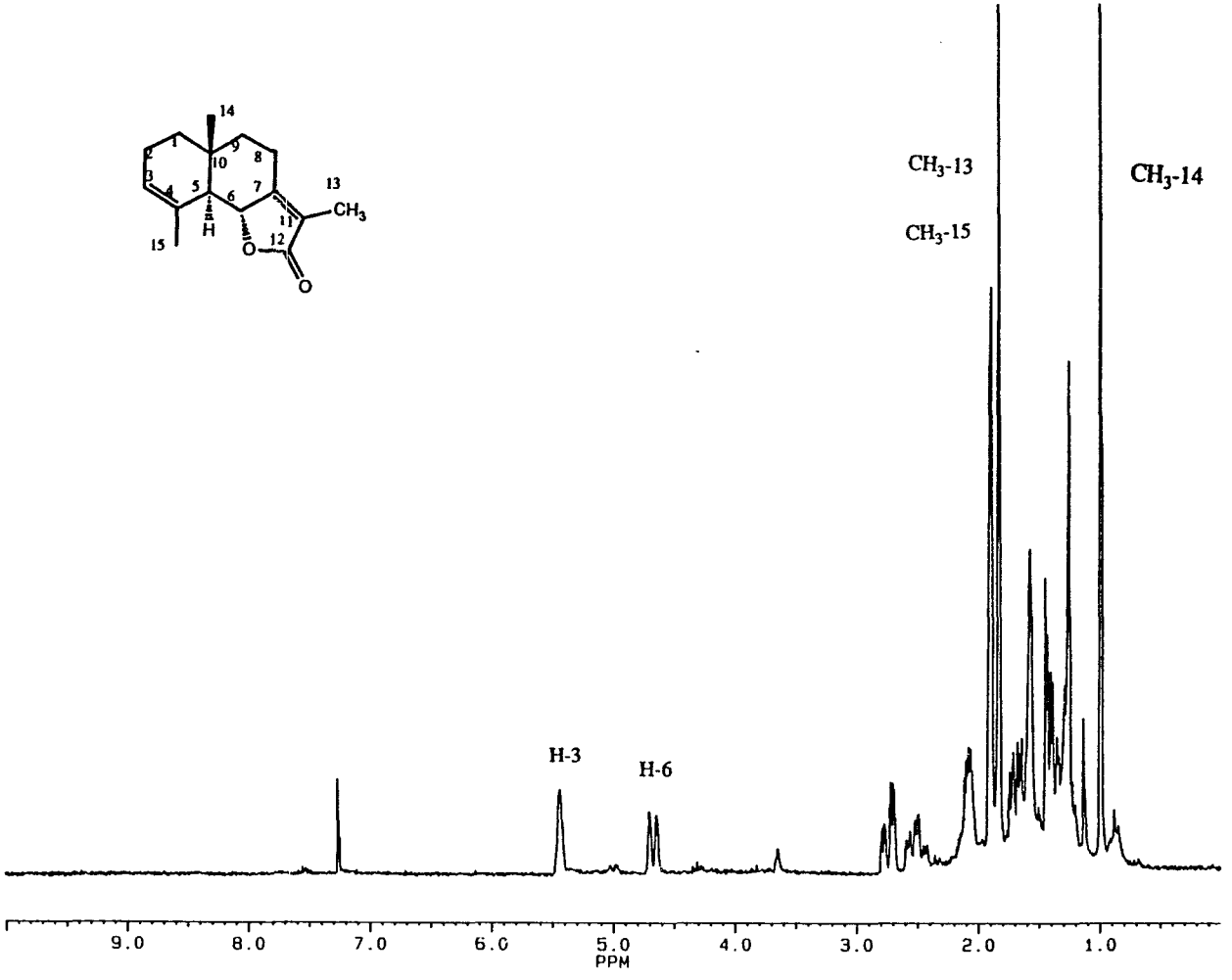


Figure 3.16. ^{1}H NMR spectrum of compound **19** in $CDCl_3$.

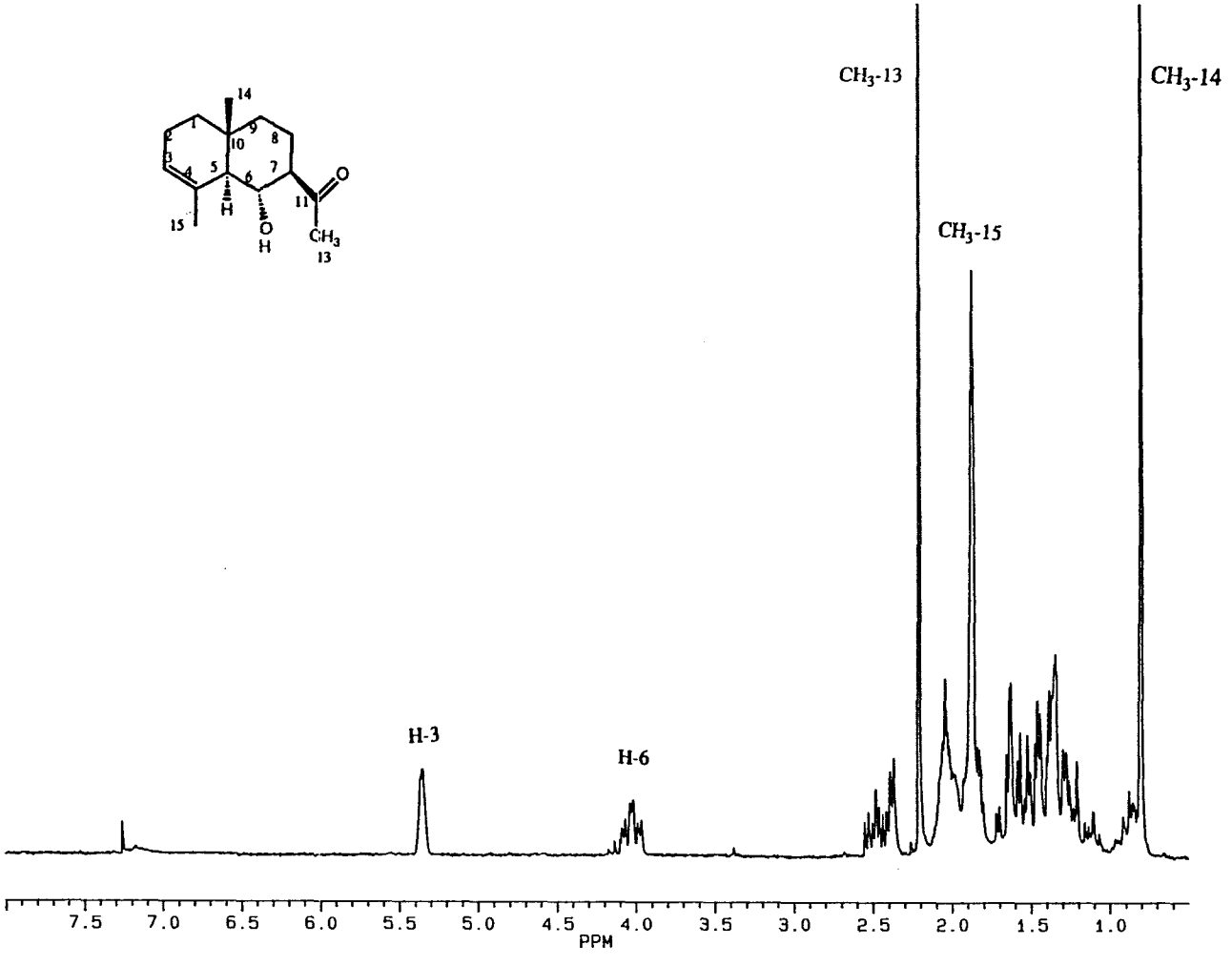


Figure 3.17. ^{1}H NMR spectrum of compound **20** in $CDCl_3$.

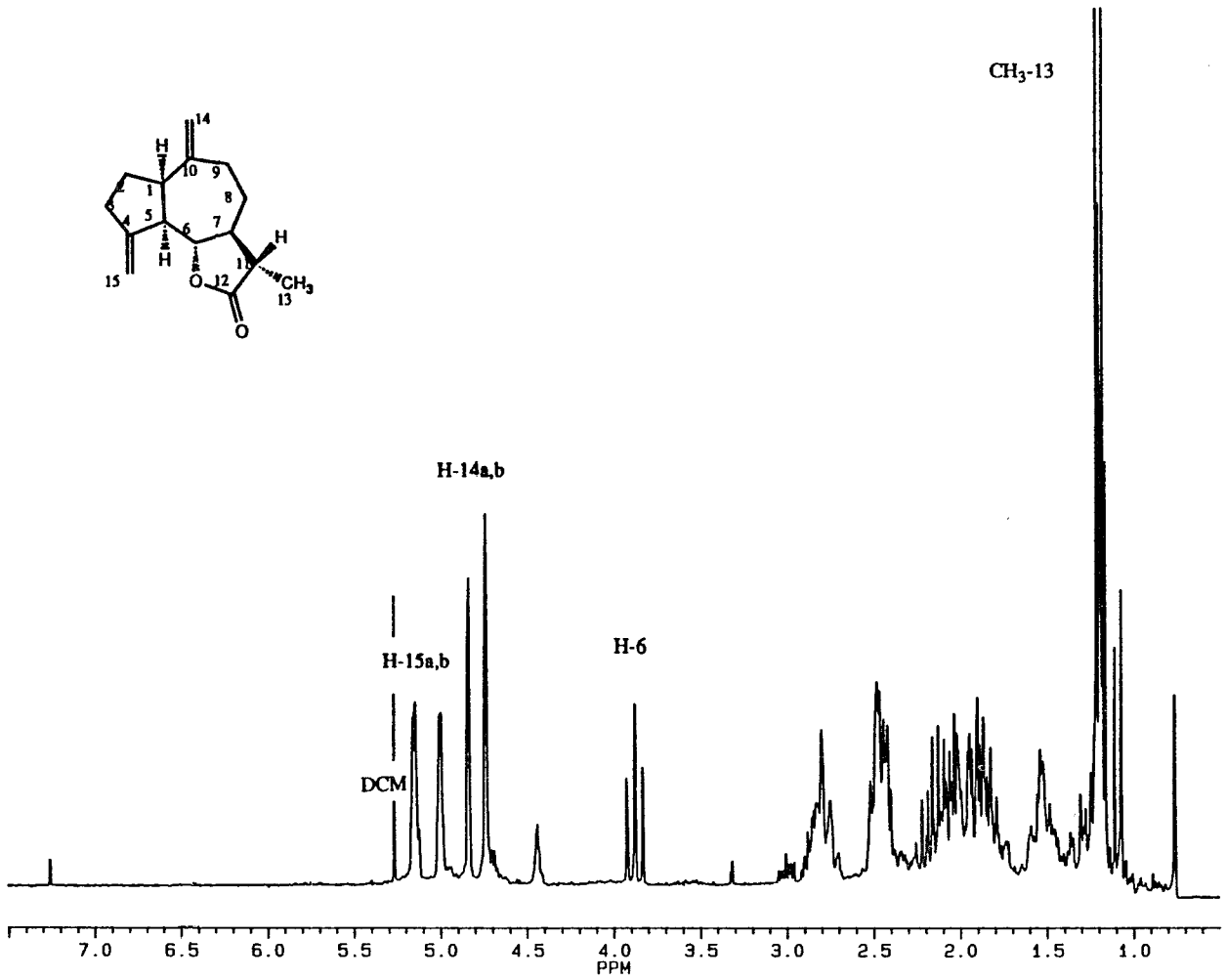


Figure 3.18. ^{1}H NMR spectrum of compound **21** in $CDCl_3$.

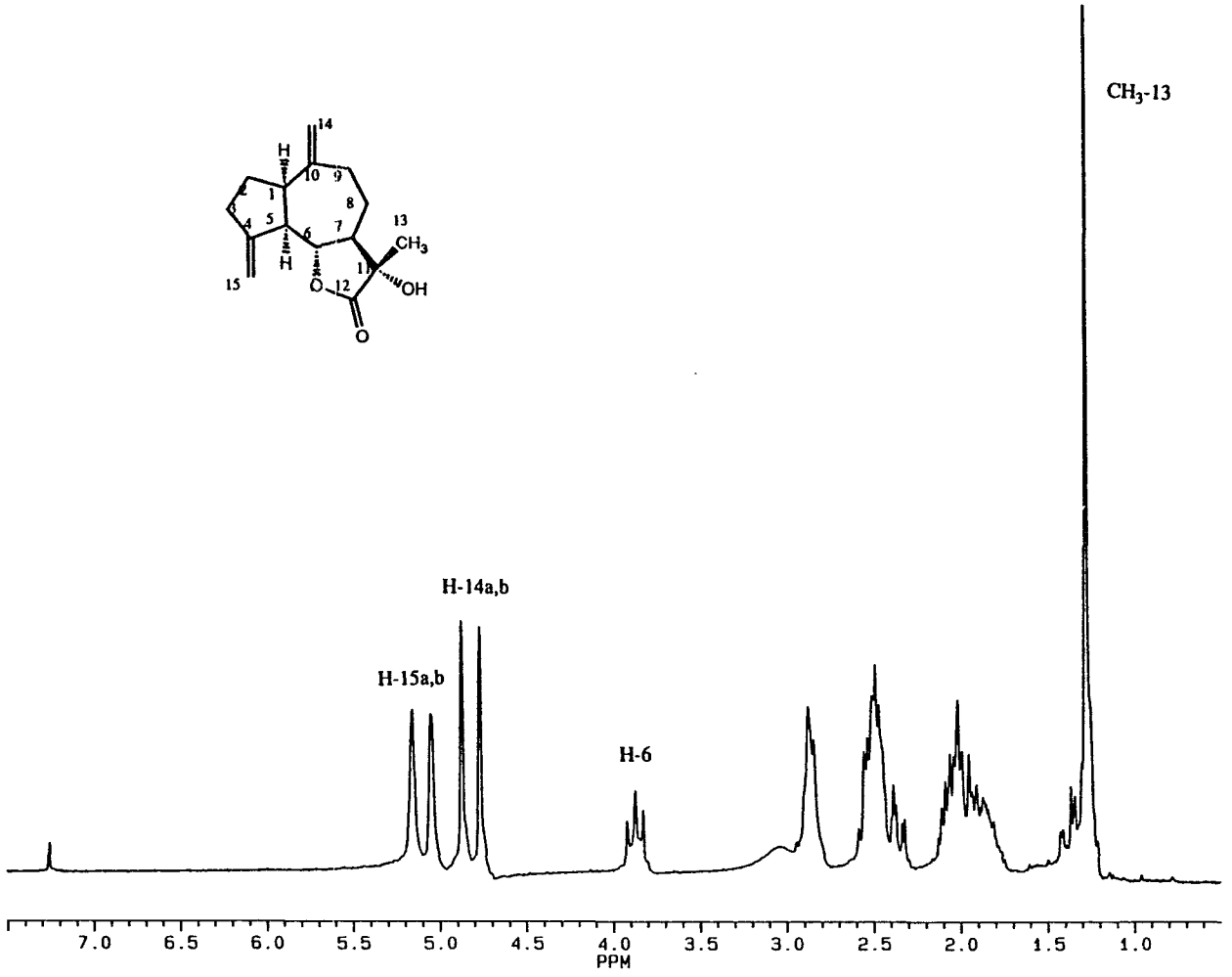


Figure 3.19. ^{1}H NMR spectrum of compound **22** in $CDCl_3$.

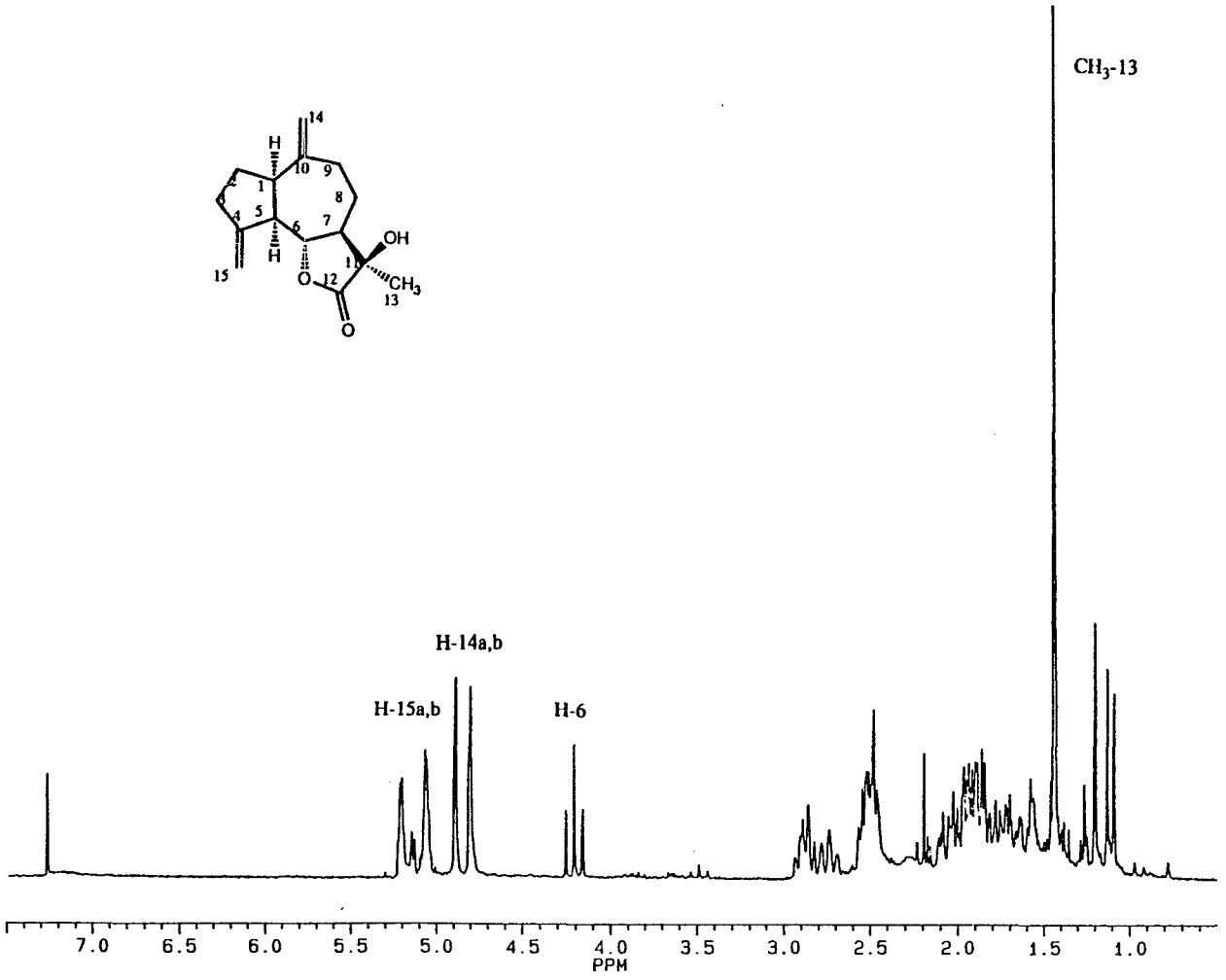


Figure 3.20. ^{1}H NMR spectrum of compound **23** in $CDCl_3$.

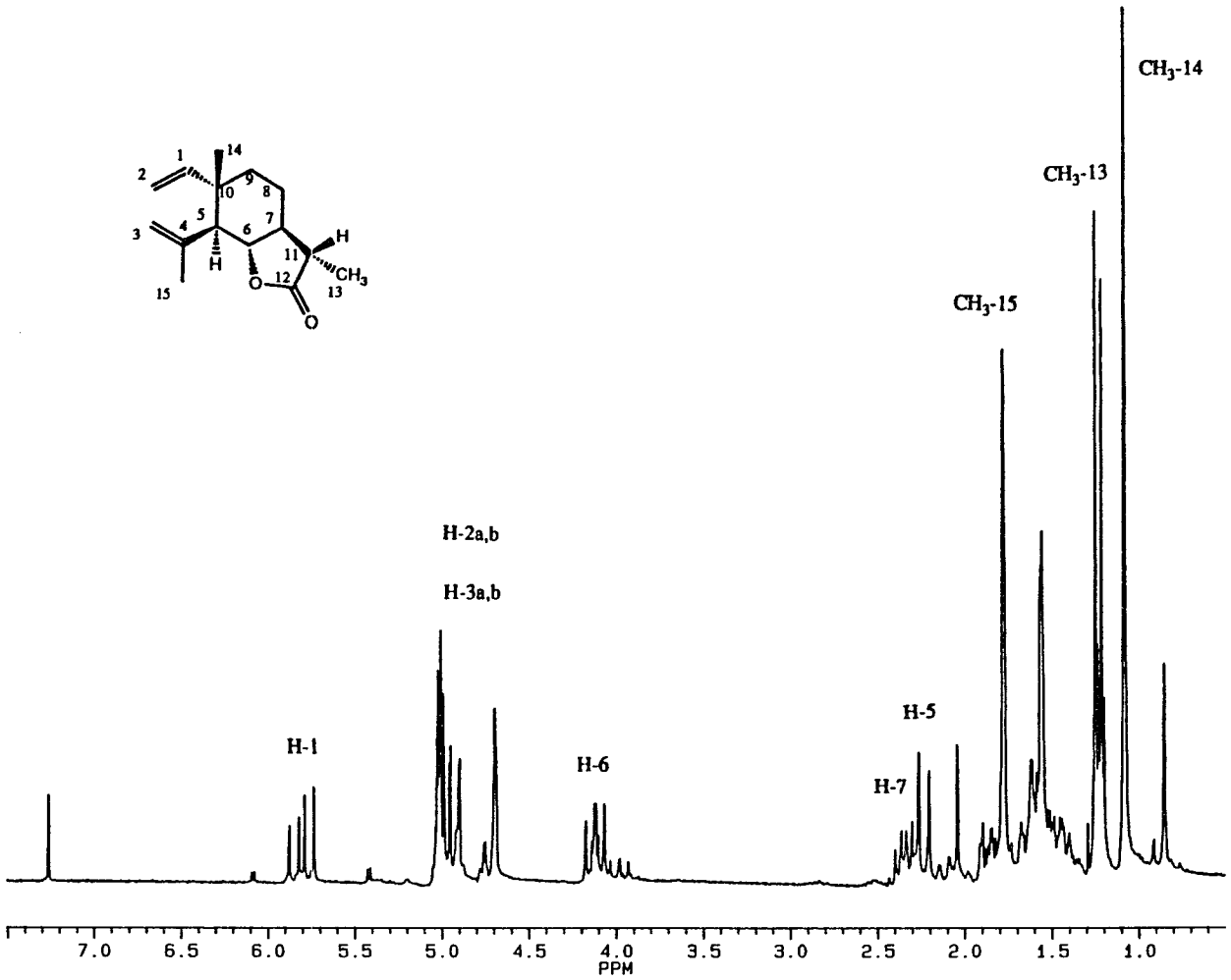


Figure 3.21. ^{1}H NMR spectrum of compound **24** in $CDCl_3$.

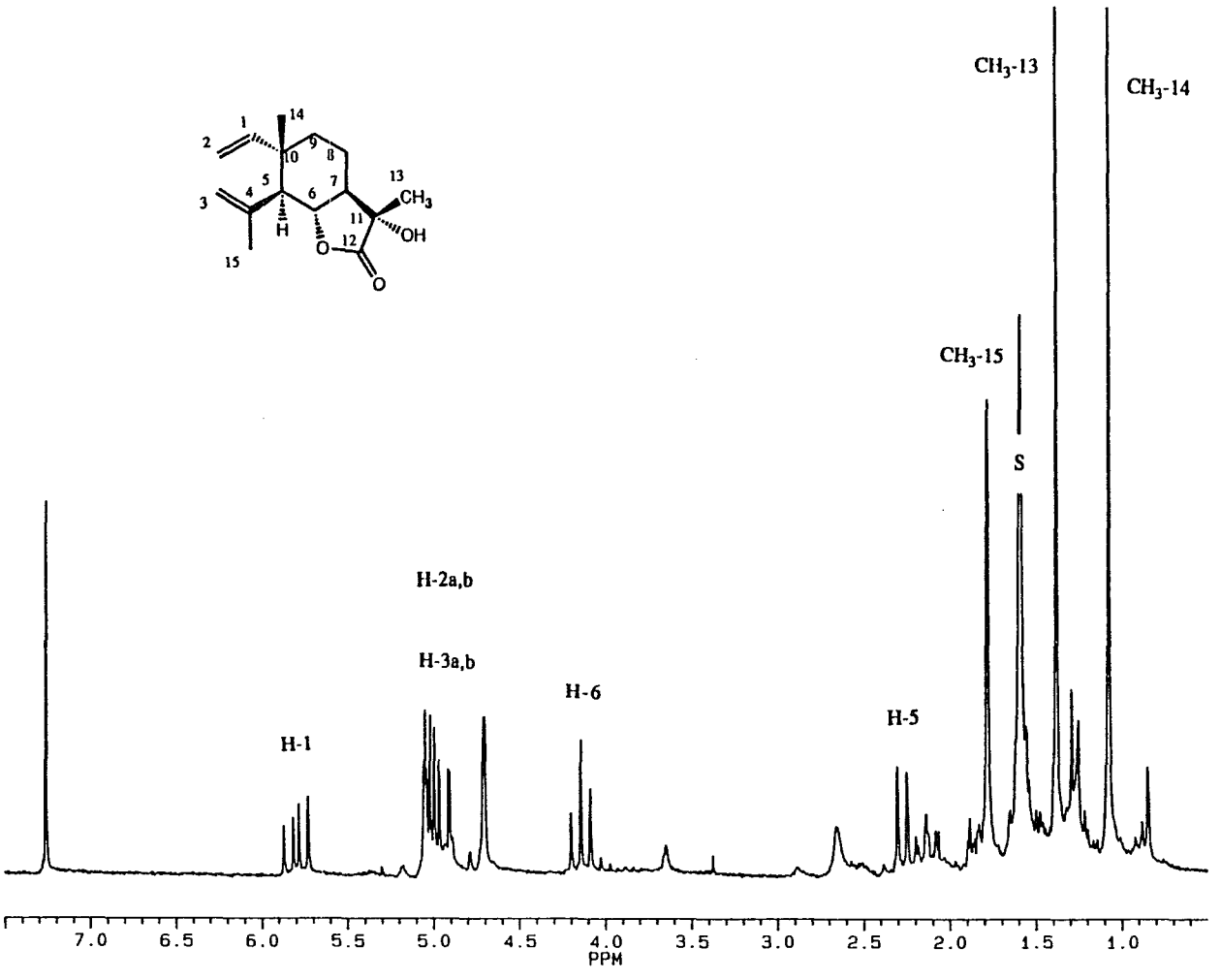


Figure 3.22. ^{1}H NMR spectrum of compound **25** in $CDCl_3$.

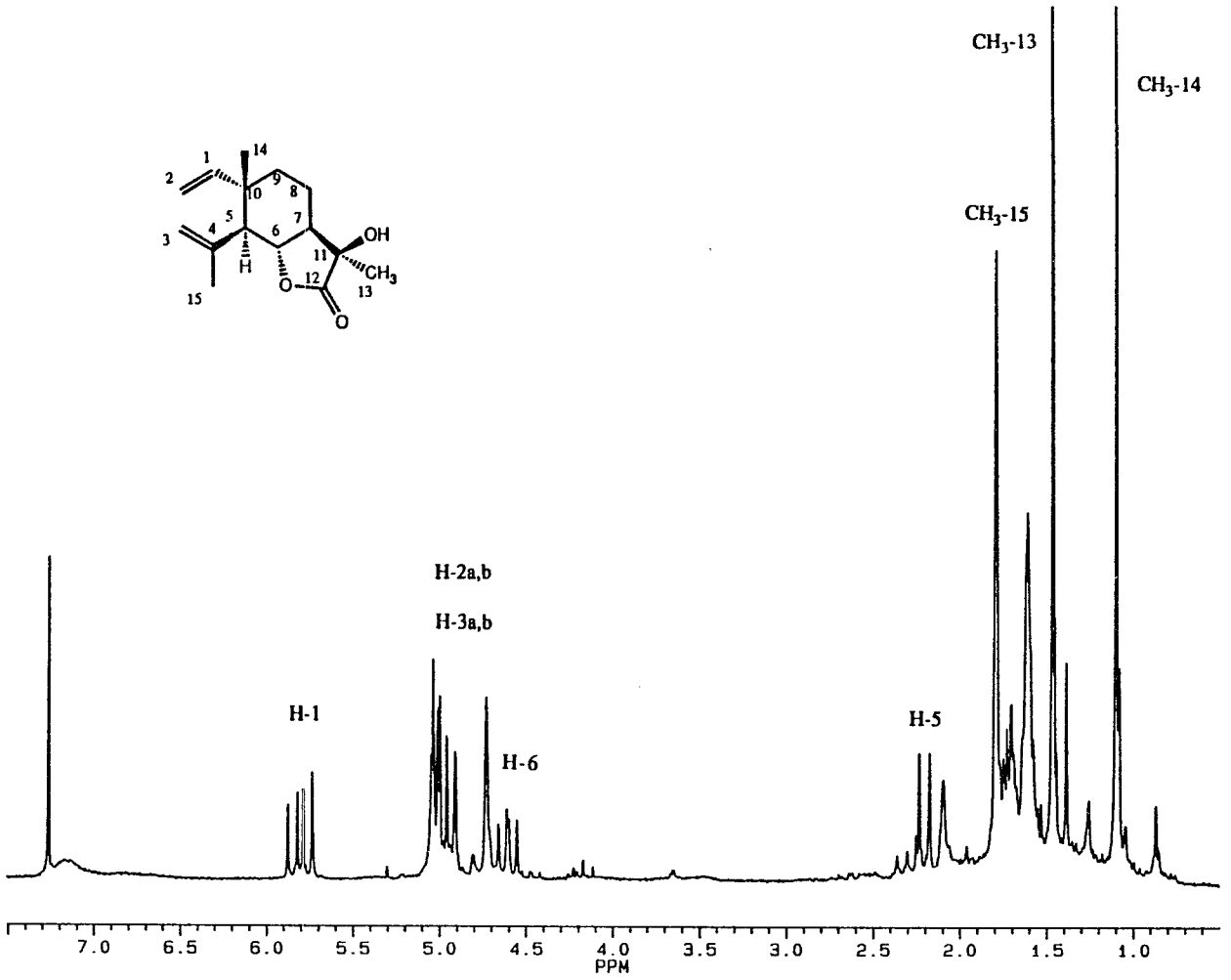


Figure 3.23. ^{1}H NMR spectrum of compound **26** in $CDCl_3$.

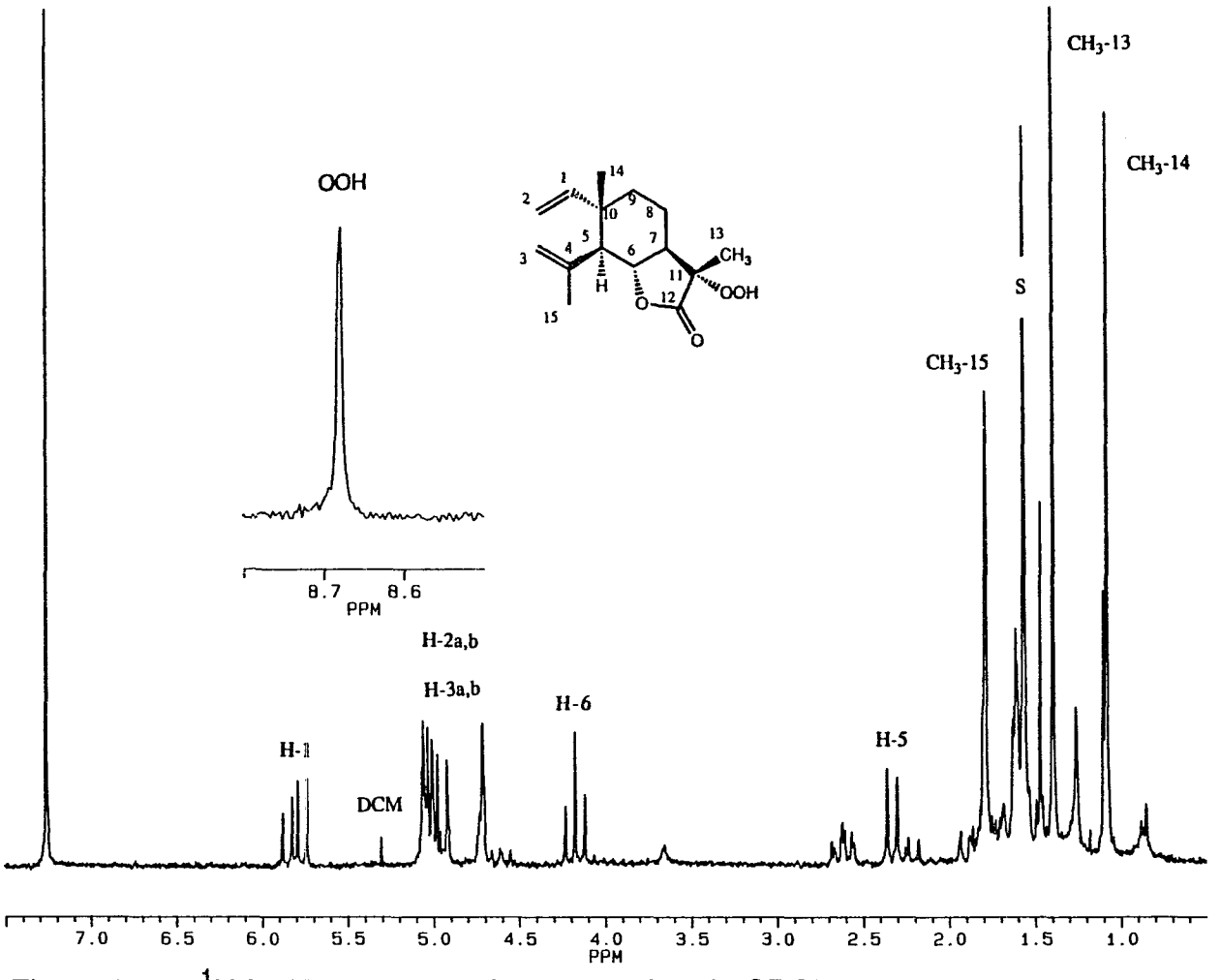


Figure 3.24. ^{1}H NMR spectrum of compound **27** in $CDCl_3$.

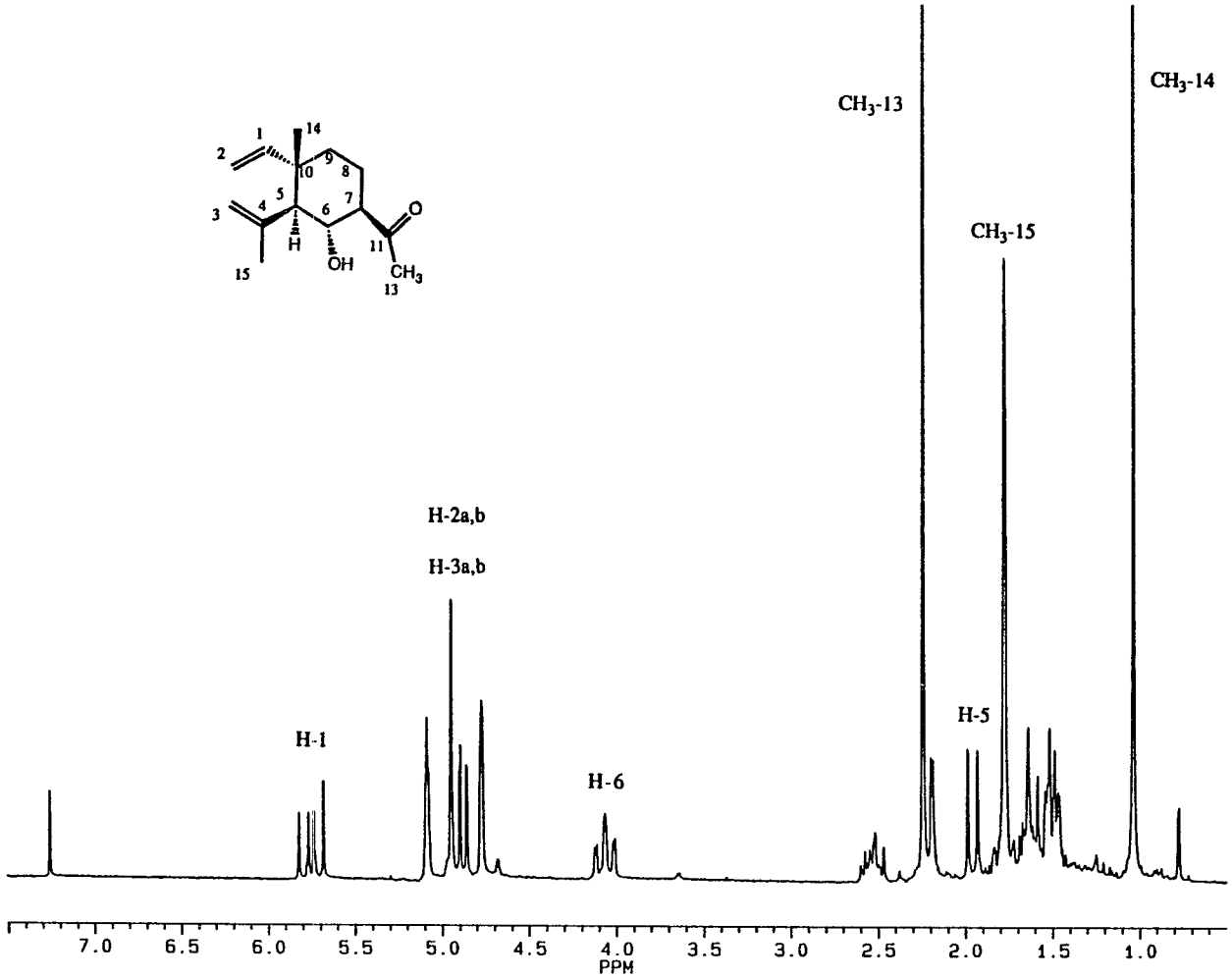


Figure 3.25. ^{1}H NMR spectrum of compound **28** in $CDCl_3$.

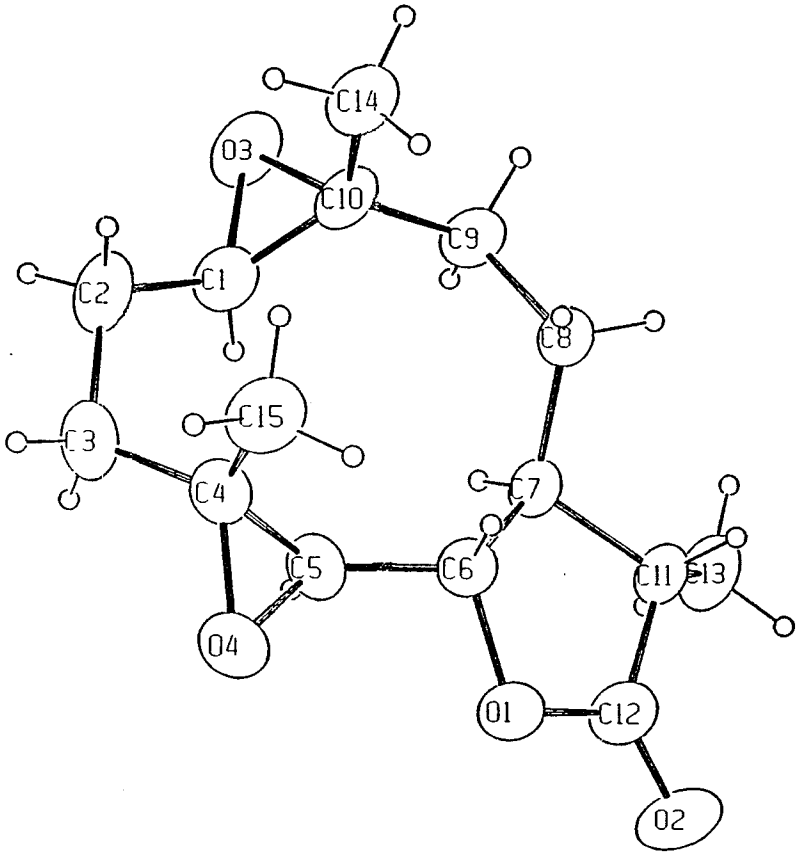


Figure 3.26. Molecular structure of compound **10**.

References

1. Fronczek, F.R.; Vargas, D.; Fischer, N.H.; Hostettmann, K. *J. Nat. Prod.* **1984**, *47*, 1036-9.
2. Vargas, D.; Fronczek, F.R.; Fischer, N.H.; Hostettmann, K. *J. Nat. Prod.* **1986**, *49*, 133-8.
3. Hostettmann, K.; Marston, A. "Plants Used in African Trtaditional Medicines." in Folk Medicine, The Art and the Science., editor R.P. Steiner, **1986**, ACS, Wash., D.C. pp. 111-24.
4. Vargas, D.; Younathan, E.S.; Fischer, N.H., unpublished results.
5. Picman, A.K. *Biochem. System. Ecol.* **1986**, *14*, 255-81.
6. Rodriguez, E.; Towers, G.H.N.; Mitchell, J.C. *Phytochemistry* **1976**, *15*, 1573-80.
7. Collado, I.G.; Macias, F.A.; Massanet, G.M.; Molinillo, J.M.G.; R.-Luis, F. *J. Org. Chem.* **1987**, *52*, 3323-6.
8. Gersmann, H.R.; Bickel, A.F. *J. Chem. Soc. (B)* **1971**, 2230-7.
9. Biloski, A.; Ganem, B. *Synthesis* **1983**, *7*, 537-8.
10. Bartlett, P.D.; Schaap, A.P. *J. Amer. Chem. Soc.* **1970**, 3223-6.
11. Rodriguez, A.A.S.; Garcia, M.; Rabi, J. *Phytochemistry* **1978**, 953-4.
12. Wu, Y.-F. "Attempts Toward a Biogenetic-like Synthesis of Pseudoguaianolides From a 4,5-Epoxy-germacranolide, Dihydroparthenolide." Thesis, Louisiana State University, **1977**.
13. Parodi, F.J. "Structure Elucidation of Natural Products From Asteraceae using Modern NMR Techniques and Biomimetic Transformations of 11,13-Dihydroparthenolide." Dissertation, Louisiana State University, **1988**.
14. Lee, I.-Y. "New Sesquiterpene Lactones from the Genera *Calea*

Berlandiera (Asteraceae) and the Fragmentation Reactions of 1,3-Dihydroxyeudesmanolide Derivatives." Dissertation, Louisiana State University, **1983**.

15. Rao, A.S.; Sadgopal, A.P.; Bhattacharyya, S.C. *Tetrahedron* **1961**, *13*, 319-23.
16. Govindachari, T.R.; Joshi, B.S.; Kamat, V.N. *Tetrahedron* **1965**, 1509-19.
17. Fischer, N.H.; Macias, F.A.; Parodi, F.; Vasquez, M.; Zinn, R. ^{13}C NMR Review of Sesquiterpene Lactones. **1989**.

Part B. Enolate Oxidations With (Camphorylsulfonyl)oxaziridine.

Improved Stereospecific Method For The Preparation of 11β–Hydroxysesquiterpene Lactones.

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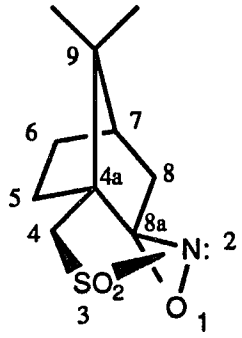
Stereospecific oxidation alpha to the lactonic carbonyl group of the sesquiterpene lactone dihydroparthenolide (DHP) has been achieved by reacting the enolate anion of DHP with a chiral oxidizing agent, (camphorylsulfonyl)oxaziridine. Oxidation with either the (+)- or the (-)-oxaziridine generates the 11β-hydroxydihydroparthenolide epimer exclusively (66-72%) with no detection of 11α-hydroxydihydroparthenolide or any decomposition product.

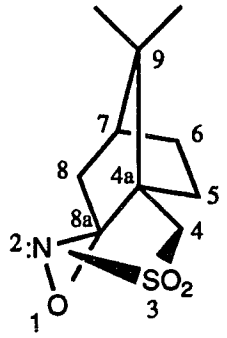
Introduction

Oxidation of the enolate anion of sesquiterpene lactones with oxygen has been recently used in the chemical transformation of sesquiterpene lactones[1] and the preparation of 11-hydroxysesquiterpene lactones as model compounds to study inhibition of the enzyme phosphofructokinase (PFK).[2] The reaction with oxygen is not stereospecific: both the 11α-hydroxy and the 11β-hydroxy-sesquiterpene lactones are isolated in yields ranging from 13-47%. Also, a 14-carbon decomposition product can often be isolated from the reaction mixture.

Davis et al.[3] reported the asymmetric oxidation of prochiral ester enolates using a chiral oxidizing agent instead of oxygen. The chiral oxidizing agents, (+)-(2R,8aS)-(camphorylsulfonyl)oxaziridine and (-)-(2S,8aR)-(camphorylsulfonyl)-oxaziridine (Scheme 3.4) are stable, easily prepared[4], commercially available



(+)-(2R,8aS)-(Camphorylsulfonyl)oxaziridine



(-)-(2S,8aR)-(Camphorylsulfonyl)oxaziridine

Scheme 3.4

(Aldrich), and generate α-hydroxy esters with yields ranging from 70-80% and enantiomeric excesses of 80-98%.[3]

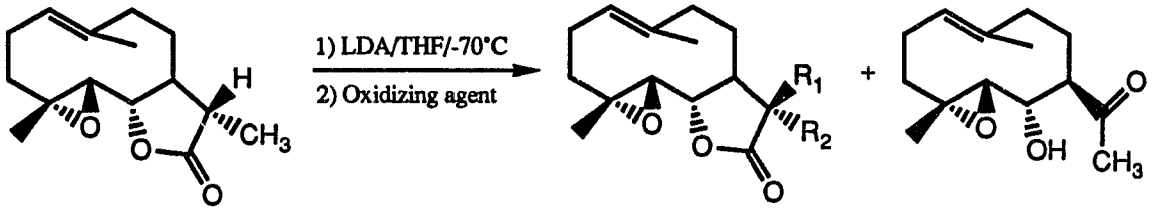
We attempted to carry out the oxidation of a sesquiterpene lactone, dihydroparthenolide (**4**), with these chiral oxidizing agents with the hope that the reaction would be stereoselective and the yields would be improved in comparison to the oxidation with oxygen (Scheme 3.5).

Results and Discussion

Oxidation of the enolate anion of DHP with (-)-(camphorylsulfonyl)oxaziridine generates the 11β-hydroxydihydroparthenolide (**6**) product in 66% yield. Neither the 11α-hydroxydihydroparthenolide (**5**) nor the possible decomposition product (**13**) were detected (Table 3.4). The same results were observed for the oxidation of the enolate anion of DHP with (+)-(camphorylsulfonyl)oxaziridine except the yield of 11β-hydroxydihydroparthenolide was slightly higher at 72%. When compared to the enolate oxidation with oxygen, clearly, using these chiral oxidizing agents is preferred because the yields are much improved and the reaction is stereospecific.

Apparently, the frozen solute conformation of DHP[4] (a 7,6-trans lactone) favors a beta-oriented attack by the oxidizing agents (both enantiomers): similar beta-oriented attacks are observed for the $NaBH_4$ reductions (in methanol) of the α-methylene group of similar sesquiterpene lactones like parthenolide (**4a**, Scheme 3.1) and costunolide (**1a**, Scheme 3.1). Enolate oxidations with (camphorylsulfonyl)oxaziridines may not be stereospecific with conformationally more flexible sesquiterpene lactones like 7,8-lactonized or 7,6-cis-lactonized germacranolides.

A key disadvantage of any chiral auxiliary used in synthesis is the necessity of



4

5 $R_1 = CH_3$, $R_2 = OH$

6 $R_1 = OH$, $R_2 = CH_3$

13

Scheme 3.5

Table 3.4. Yield of Products From Enolate Oxidations of Dihydroparthenolide (DHP)[a]

	% Yield		
Oxidizing agent	11α-OH-DHP	11β-OH-DHP	Decomposition
(-)-oxaziridine	---	66	---
(+)-oxaziridine	---	72	---
oxygen	29	18	16

a = Yields are based on recovered starting material

preparing and eventually removing the chiral auxiliary reagent. Davis and Haque[6] report that the best way to remove unreacted oxaziridine or its corresponding sulfonylimine ($RSO_2N=CR_2$) is by precipitation from diethyl ether at -78°C. Use of this procedure on the product mixtures of the enolate oxidations of DHP with the oxaziridines always resulted in removal of only 60-70% of the chiral auxiliary or its reduced form (sulfonylimine). Our attempts to separate these reagents from the products by size-exclusion chromatography (Sephadex-LH) were not successful. Better separation was achieved by dry column (silica gel) chromatography[7] eluting with DCM/acetone (9:1). The unreacted oxaziridine and its sulfonylimine elute in the very early fractions.

The chiral auxiliary can be regenerated from its sulfonylimine by oxidation with potassium peroxymonosulfate (Dupont Oxone).[8]

Experimental Section

(-)-(2S,8aR)-(Camphorylsulfonyl)oxaziridine and (+)-(2R,8aS)-(camphorylsulfonyl)oxaziridine were purchased from Aldrich and used without further purification. Reagent grade tetrahydrofuran (THF) was freshly distilled over Li metal before use to remove any traces of water. A 1.5M solution of lithium diisopropylamide (LDA) in cyclohexane (Aldrich) was used without further purification. ^1H NMR spectra were recorded on a Bruker-AC200 spectrometer in $CDCl_3$ using $SiMe_4$ as an internal standard.

Oxidation of the enolate anion of 11,13-dihydroparthenolide with (-)-(2S,8aR)-(camphorsulfonyl)oxaziridine. Dihydroparthenolide **(4)** (200mg, 0.8mmol) dissolved in 5ml of dry THF was added slowly over 15min. by syringe to a stirred solution of 0.7ml (1.04mmol) of LDA in 5ml of THF under

argon at -70°C. After stirring the solution for an additional 15 minutes, a THF solution of (-)-(2S,8aR)-(camphorylsulfonyl)oxaziridine (370mg, 1.6mmol) was added to the reaction flask by syringe over a 5 minute period at -70°C. After an additional 5 minutes, the reaction was quenched with the addition of 5ml of a saturated aqueous NH_4Cl solution. The reaction mixture was extracted with diethyl ether (6 x 10ml). The ether solution was dried over anhydrous Na_2SO_4, filtered, and the solvent was evaporated.

TLC analysis (silica gel, eluting with DCM/acetone; 9:1) of the product mixture showed some unreacted starting material, which appeared as a dark red spot with R_f = 0.65 after spraying with $CoCl_2/H_2SO_4$ and applying heat, and a large red spot (R_f = 0.45). Unreacted (-)-(camphorylsulfonyl)oxaziridine and its reduced form camphorylsulfonylimine are not visible by TLC either under UV light or after spraying with $CoCl_2/H_2SO_4$ and applying heat. Towson et al.[8] report the TLC separation and visualization of the oxaziridine and the corresponding imine by eluting with DCM and developing with 10% molybdophosphoric acid in ethanol and heating (imine R_f = 0.28, oxaziridine R_f = 0.62).

Attempted precipitation of the oxaziridine and the imine at -78°C in diethyl ether only removed 60-70% of these reagents. Repeated precipitations did not further purify the product. Attempts to separate the oxidant and its reduced form by size-exclusion chromatography (Sephadex-LH) eluting with methanol/DCM (1:1) was not successful. Dry column (silica gel) chromatography[7] was finally used to separate the product mixture eluting with DCM/acetone (9:1). The oxaziridine and the imine eluted in the very early fractions. DHP (58mg) was recovered and 100mg (66%) of 11β-hydroxydihydroparthenolide (**6**) was isolated. The ^1H NMR data for compound **6** was identical to the data for the product isolated from the reaction of the enolate anion of DHP with oxygen. No decomposition product (**13**) was

isolated and no 11α-hydroxydihydroparthenolide (**5**) was found.

Oxidation of enolate anion of DHP with (+)-(2R,8aS)-(camphorylsulfonyl)oxaziridine. Dihydroparthenolide (200mg) was oxidized as before with (+)-(2R,8aS)-(camphorylsulfonyl)oxaziridine. The product mixture was separated by dry column (silica gel) chromatography[7] eluting with DCM/acetone (9:1). DHP (56mg) was recovered and 110mg (72%) of 11β-hydroxydihydroparthenolide (**6**) was isolated as the only product.

References

1. Collado, I.G.; Macias, F.A., Massanet, G.M.; Molinillo, J.M.G.; R.-Luis, F. *J. Org. Chem.* **1987**, *52*, 3323-6.
2. Pentes, H.; Fischer, N.H.; Macias, F. unpublished results.
3. Davis, F.A.; Hague, M.J.; Ulatowski, T.G.; Towson, J.C. *J. Org. Chem.* **1986**, *51(12)*, 2402-4.
4. Takeda, K. *Tetrahedron* **1974**, *30*, 1525-34.
5. Rao, A.S.; Kelkar, G.R.; Bhattacharyya, S.C. *Tetrahedron* **1960**, *9*, 275-83.
6. Davis, F.A.; Haque, M.S. *J. Org. Chem.* **1986**, *51*, 4085-7.
7. Loev, B.; Goodman, M. M. *Chem. and Ind.* **1967**, 2026-32.
8. Towson, J.C.; Weismiller, M.C.; Lal, G.S.; Sheppard, A.C.; Davis, F.A. *Organic Synthesis* **1990**, *69*, 158-67.

Chapter 4. Synthesis of Sesquiterpene Lactones.

Part A. Synthesis of 11β,15-Dihydroxysaussurea Lactone From Costunolide

Chemical Transformation of Costunolide into the Natural Compound 11β,15-Dihydrosaussurea Lactone

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11β,15-Dihyroxysaussurea lactone **(11)**, a secondary metabolite isolated from *Centaurea castellana* Boiss, has been regioselectively synthesized from costunolide **(1)**. This transformation involves a Cope rearrangement, allylic oxidation with selenium dioxide, and an enolate oxidation with gaseous oxygen.

Introduction

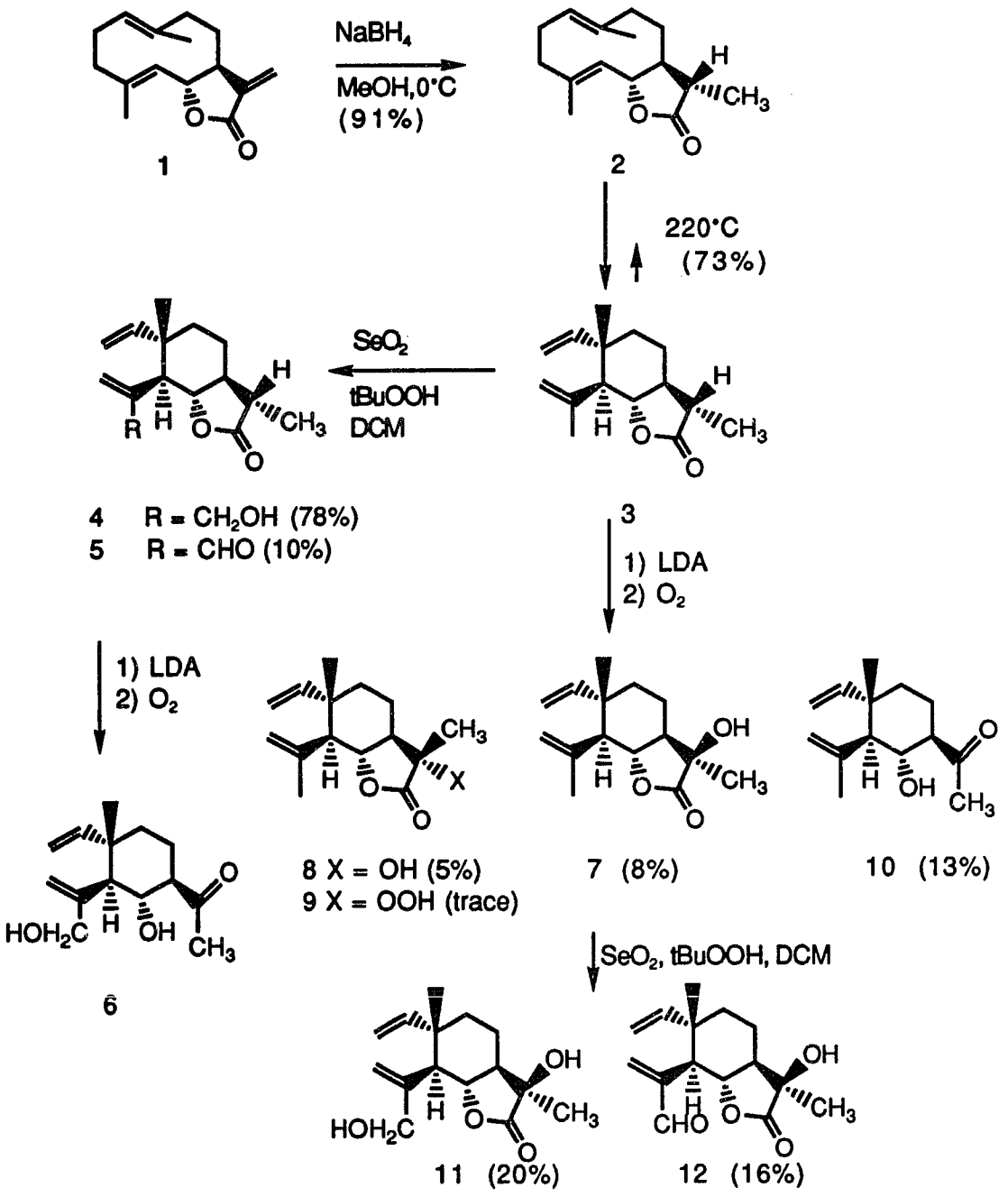
Oxidation at the 7- or 11- position of sesquiterpene lactones is uncommon in nature. However, 7-hydroxysesquiterpene lactones exhibit some very interesting biological activities.[1] 7-Hydroxysesquiterpene lactones have been shown to be potent molluscicidal compounds and inhibitors of phosphofructokinase (PFK).[1] It has been proposed that these compounds are effective PFK inhibitors because they mimic the action of sugar molecules and can therefore bind to the active site of the enzyme. 11-Hydroxysesquiterpene lactones may also mimic sugar molecules and thereby be effective PFK inhibitors. These proposals prompted us to carry out the synthesis of a naturally occurring 11-hydroxysesquiterpene lactone, 11β,15-dihydroxysaussurea lactone **(11)** which was isolated by Gonzalez et al. from the aerial parts of *Centaurea castellana* Boiss.[2] The starting material for this transformation was the readily available sesquiterpene lactone costunolide **(1)**.[3]

Oxidation at C-15 was achieved by selenium dioxide oxidation in the presence of tert-butyl hydroperoxide. Oxidation at the 11- position was achieved by trapping the enolate anion with gaseous oxygen.

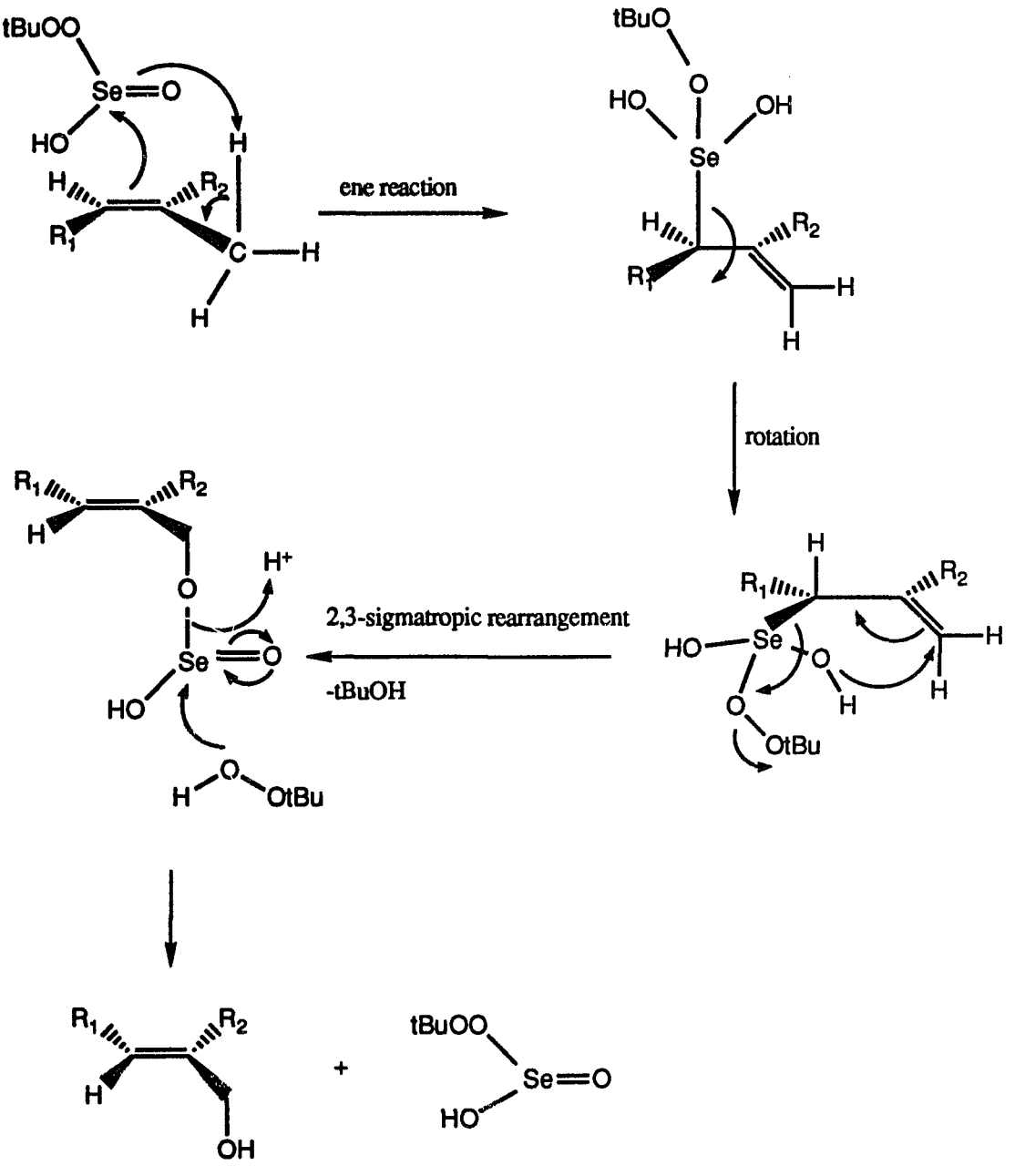
Results and Discussion

Costunolide (**1**) was isolated by silica gel vacuum liquid chromatography (VLC) from Costus Resinoid (Pierre Chauvet, S.A.). The white, crystalline solid isolated exhibited physical and spectral properties identical to that already reported in the literature for this compound.[3] The exocyclic methylene group of costunolide (**1**) was reduced using $NaBH_4$ in methanol at 0°C (Scheme 4.1) giving a 91% yield of dihydrocostunolide (**2**).[4] The Cope rearrangement of **2** to saussurea lactone (**3**) was carried out at 220°C for 3 minutes. The spectroscopic and physical data for compound **3** isolated from the product mixture was in good agreement with the data already reported in the literature for saussurea lactone (**3**).[5] The equilibrium for this Cope rearrangement favors the formation of the elemanolide (saussurea lactone) by a molar ratio of 1.8/1.0 or 64%/36%. The yield of **2** was 52% (73% based on recovered **2**) and can be improved by isolating **2** from the product mixture and repeating the Cope rearrangement.

Saussurea lactone (**3**) was regioselectively oxidized at the C-15 allylic position (Sharpless allylic oxidation conditions)[6] by reaction with 0.5mol. equiv. of selenium dioxide and 2mol. equiv. of 70% tert-butyl hydroperoxide in dichloromethane at room temperature. It has been proposed that the mechanism of this reaction involves three steps: an ene reaction between the allylic compound and the selenium tert-butyl hydroperoxide complex, a 2,3-sigmatropic rearrangement, followed by hydrolysis of the resulting seleninic ester (Scheme 4.2).[7] Apparently, oxidation does not occur at C-5 of saussurea lactone because the conformation of


NaBH4
MeOH,0°C
(91%)
1
2
220°C
(73%)
SeO2
tBuOOH
DCM
3
4 R = CH2OH (78%)
5 R = CHO (10%)
1) LDA
2) O2
8 X = OH (5%)
9 X = OOH (trace)
7 (8%)
10 (13%)
6
SeO2, tBuOOH, DCM
11 (20%)
12 (16%)

Scheme 4.1


tBuOO
Se=O
HO
H
H
R2
R1
C—H
H
ene reaction
tBuO
O
HO
OH
Se
H
R2
R1
H
H
rotation
R1
R2
H
O
H+
Se=O
HO
2,3-sigmatropic rearrangement
-tBuOH
H
O
OtBu
H
R1
R2
HO
Se
O
H
H
O
H
OtBu
R1
R2
H
OH
+
tBuOO
Se=O
HO

Scheme 4.2

saussurea lactone necessary for the ene reaction at C-5 requires that H-5 be perpendicular to the C3-C4 double bond. Molecular models show that with this requirement, there exists significant steric hindrance between the vinyl protons H-2 and the vinyl protons H-3 or the methyl group CH_3-15. No such steric inhibition is present in the transition state for the ene reaction at the C-15H's, so oxidation occurs here preferentially. The major product of the reaction is the C-15 alcohol, 8-deoxymelitensin (**4**) (78%) and a minor product is the C-15 aldehyde (**5**) (10%) which results from further oxidation of **4** under the reaction conditions. The spectral and physical data of **4** is identical to the 8-deoxy-derivative of a natural compound, melitensin, which was synthesized by Ando et al.[8] by a less effecient route from α-santonin. The aldehyde (**5**) was characterized by two IR carbonyl absorptions at 1771cm^{-1} (lactone) and 1689cm^{-1} (conjugated aldehyde) and the ^1H NMR spectrum which showed the disapperance of the singlet for CH_3-15 and the appearance of an aldehydic signal at 9.43ppm. Also, the ^{13}C spectrum showed the presence of an aldehyde resonance at 193.8ppm.

In order to synthesize the target molecule (**11**), an attempt was made to oxidize **4** at the 11-position by trapping the enolate anion of **4** with gaseous oxygen.[9] The only product isolated from this reaction was the decomposition product **6** in which one carbon atom has been lost (probably C-12) through decarboxylation of the hydroperoxy-anion intermediate. These decomposition products are often found in reactions of this type;[10] their mechanism of formation are as of yet unknown. Compound **6** was characterized by its IR spectrum which showed the presence of a ketone carbonyl absorption at 1708cm^{-1} instead of a lactone absorption. This was further verified by ^1H NMR data which showed the appearance of a methyl singlet at 2.23ppm, indicating a methyl ketone.

To synthesize the target molecule **11**, it was then attempted to oxidize the

enolate anion of **3** first which would then be followed by allylic oxidation with selenium dioxide. Reaction of **3** with lithium diisopropylamide (LDA) then oxygen generated 4 products: 11β-hydroxysaussurea lactone (**7**) (8%), 11α-hydroxysaussurea lactone (**8**) (5%), a trace amount of 11α-hydroperoxysaussurea lactone (**9**), and the decomposition product (**10**) (13%). This reaction is not stereospecific and gives reproducibly low yields on this substrate. The four products (**7,8,9,10**) were isolated from the crude reaction mixture by column chromatography. Compounds **7** and **8** show OH absorptions in the infrared region at 3440-3450cm^{-1}. Their ^{1}H NMR spectra clearly show the collapse of the CH_3-13 doublet to a singlet with a downfield shift of ~0.2ppm. The 11-hydroxysaussurea lactone epimers were distinguished by the chemical shift of H-6 in their ^{1}H NMR spectra. The chemical shift of H-6 of 11β-hydroxysaussurea lactone is shifted downfield (~0.5ppm) compared to H-6 of saussurea lactone due to a deshielding effect of the β-hydroxyl group. This shift of H-6 is not observed for the 11α-hydroxy-epimer. The ^{1}H NMR data of the hydroperoxide (**9**) shows a singlet at 8.68ppm (OOH), a methyl singlet at 1.39ppm (C_{13}-CH_3), and no shift in H-6 (relative to saussurea lactone) indicating the 11α-hydroperoxy-derivative. The IR spectrum of the decomposition product (**10**) shows a ketone carbonyl absorption at 1708cm^{-1} and the ^{1}H NMR data shows a methyl singlet at 2.25ppm (C_{13}-CH_3) indicating a methyl ketone.

The selenium dioxide oxidation of 11β-hydroxysaussurea lactone (**7**) was carried out and generated the natural compound (**11**) (20%) and also the corresponding C-15 aldehyde (**12**) (16%). The spectral data for **11** was identical to that previously reported for the natural compound.[2] The aldehyde (**12**) was characterized by two carbonyl absorptions at 1752cm^{-1} (lactone) and 1691cm^{-1} (conjugated aldehyde) and its ^{1}H NMR spectrum which showed the disappearance

of the methyl singlet (C_{15}-CH_3) and the appearance of an aldehydic proton resonance at 9.44ppm.

Thus, the regioselective synthesis of 11β,15-dihydroxysaussurea lactone has been achieved in 4 steps with an overall yield of 1%.

Experimental Section

^1H NMR spectra were recorded on a Bruker-AC200 spectrometer in $CDCl_3$ using Me_4Si as an internal standard. Mass spectra were recorded on a HP5985 spectrometer. Infrared spectra were recorded on a Perkin-Elmer 1760x spectrometer in film on NaCl plates. Column chromatographic separations were made on silica gel (60-200M, J.T. Baker Chemical Co.). Preparative TLC separations were made on SIL G-100(or 50) Sybron/Brinkmann plates.

The ^1H NMR (Fig. 2.1), ^{13}C NMR (Fig. 2.2), IR, and MS data for costunolide (**1**) are reported in Chapter 2, Part A, Experimental Section.

Dihydrocostunolide (2) Sodium borohydride ($NaBH_4$) (278mg, 7.05mmol) was added to a magnetically stirred solution of costunolide (**1**) (1.1g, 4.7mmol) in 10ml of methanol at 0°C. The solution was stirred for 1hr, then neutralized with 5% HCl, concentrated, diluted with water, then extracted with DCM. The DCM solution was dried over anhydrous Na_2SO_4, filtered, and evaporated yielding 1.0g (91%) of dihydrocostunolide (**2**) as white crystals. Experimental melting point 75-77°C (lit. m.p. 77-78°C).[4] The ^1H NMR (Fig. 3.1), ^{13}C NMR assignments (Table 3.3), IR, and MS data for dihydrocostunolide (**2**) are reported in Chapter 3, Part A, Experimental Section.

Cope rearrangement of Dihydrocostunolide (2) to Saussurea lactone (3). Dihydrocostunolide (**2**) (700mg, 2.99mmol) was placed in a single-neck round-bottom flask equipped with a stopcock valve and a rubber septum. The

flask was evacuated and purged with argon three times. The flask was then heated in a mineral oil bath at ~220°C for 3 minutes. TLC analysis (silica gel, eluting with 85/15 hexane/ethyl acetate) of the product mixture showed two main components: saussurea lactone (**3**), which appears as an orange spot (after spraying with $CoCl_2/H_2SO_4$ and applying heat) with R_f = 0.40 and dihydrocostunolide (**2**), which appears as a grey spot (after spraying with $CoCl_2/H_2SO_4$ and applying heat) with R_f = 0.35. Dry column (Silica Gel 60-200M, J.T. Baker) chromatography[11] was used to separate saussurea lactone (**3**) from dihydrocostunolide (**2**) by eluting with 85/15 hexane/ethyl acetate. 363mg (1.55mmol) of saussurea lactone (**3**) was isolated (52% yield, 73% based on recovered **2**) and 200mg (0.85mmol) of dihydrocostunolide (**2**) was recovered. The recovered **2** was subjected to the Cope reaction again (as before), from which an additional 62mg of **3** was isolated. The ^1H NMR (Fig. 3.21), IR, and MS data for saussurea lactone (**3**) are reported in Chapter 3, Part A, Experimental Section.

Allylic oxidation of saussurea lactone (3) to 8-deoxymelitensin (4) and 15-oxo-saussurea lactone (5). Selenium dioxide (59mg, 0.54mmol) was transferred to a round-bottom flask containing 10ml of DCM. tert-Butyl hydroperoxide (70% solution, 0.29ml, 2.14mmol) was added to the flask by syringe. This solution was stirred with a magnetic stir bar at room temperature. After 15 minutes, a DCM solution (10ml) of saussurea lactone (**3**) (250mg, 1.07mmol) was added dropwise by pipet to the flask. The reaction mixture was stirred for 24hrs., then filtered and the solvent was evaporated. The products were separated by dry column (silica gel) chromatography[11] eluting with 75/25 hexane/ethyl acetate. 111mg of unreacted saussurea lactone was recovered. 15mg (10%, based on recovered **3**) of aldehyde (**5**) was isolated as a minor product and 116mg (78%, based on recovered **3**) of alcohol (**4**) was isolated as the major

product. The reaction can be easily monitored by TLC (silica gel, eluting with 75/25 hexane/ethyl acetate). After spraying with $CoCl_2/H_2SO_4$ and applying heat, **3** appears as an orange spot ($R_f = 0.70$), **5** appears as a brown spot ($R_f = 0.51$), and **4** appears as a pink spot ($R_f = 0.23$).

8-deoxymelitensin (4) IR 3458, 1770, 1630cm^{-1}; ^{1}H NMR (Fig. 4.1): δ 5.77 (dd,1H,C_1-H,J=11,17Hz), 5.36 (s,1H,C_3-Hb), 4.96 (m,3H,C_2-Ha,b,C_3-Ha), 4.14 (dd,1H,C_1-H,J=10,12Hz), 4.04 (m,2H,C_{15}-H), 2.35 (d,1H,C_5-H,J=12Hz), 1.23 (d,3H,C_{13}-CH_3),J=7Hz), 1.09 (s,3H,C_{14}-CH_3); MS *m/z* (relative intensity) 250 (M^+) (0.6), 220 (M-30$^+$) (1.0), 207 (M-43$^+$) (0.4), 159 (M-91$^+$) (0.3).

15-oxo-saussurea lactone (5) IR 1771, 1689cm^{-1}; ^{1}H NMR (Fig. 4.2): δ 9.43 (s,1H,C_{15}-H), 6.23 (m,2H,C_3-Ha,b), 5.68 (dd,1H,C_1-H,J=11,17Hz), 4.86 (m,2H,C_2-Ha,b), 4.31 (dd,1H,C_6-H,J=10,11Hz), 3.00 (d,1H,C_5-H,J=12Hz), 1.23 (d,3H,C_{13}-CH_3,J=6Hz), 1.02 (s,3H,C_{14}-CH_3); ^{13}C NMR δ 193.8, 178.7, 146.6, 145.8, 137.1, 112.0, 79.9, 52.3, 46.4, 42.5, 41.6, 39.0,23.5, 16.9, 12.5; MS *m/z* (relative intensity) 248 (M^+) (1.3), 219 (M-29$^+$) (0.3), 215 (M-33$^+$) (0.4), 192 (M-56$^+$) (0.6).

Attempted enolate oxidation of 8-deoxymelitensin (4). Compound **4** (116mg, 0.46mmol) dissolved in 5ml of dry THF (freshly distilled over Li metal) was added slowly over 15min. by syringe to a stirred solution of 0.68ml (1.02mmol) of lithium diisopropylamide (LDA, 1.5M solution in cyclohexane, Aldrich) in 5ml of THF under argon at -70°C. After an additional 20 minutes, dry oxygen (passed over P_2O_5) was bubbled through the solution for 20 minutes at 0°C. The reaction was quenched by the addition of 3ml of distilled water. The solution was carefully neutralized with 0.5N HCl, and then extracted with diethyl ether. The ether solution was dried over anhydrous Na_2SO_4, filtered, and the

solvent was evaporated. The crude product mixture was separated by dry column (silica gel) chromatography[11] eluting with 50/50 hexane/ethyl acetate. 27mg of compound **4** was recovered and 27mg (32%) of the decomposition product **(6)** was isolated. **Ketone (6)** IR 3414, 1708, 1630cm^{-1}; ^{1}H NMR (Fig. 4.3): δ 5.68 (dd,1H,C_1-HJ=11,17Hz), 5.35 (s,1H,C_3-Hb), 4.99 (s,1H,C_3-Ha), 4.88 (m,2H,C_2-Ha,b), 4.07 (dd,1H,C_6-H,J=11Hz), 4.01 (m,2H,C_{15}-H), 2.23 (s,3H,C_{13}-CH_3), 1.02 (s,3H,C_{14}-CH_3); MS *m/z* (relative intensity) 238 (M^+) (0.03), 210 (M-28$^+$) (0.01), 205 (M-30$^+$) (0.05), 191 (M-47$^+$) (0.2), 177 (M-61$^+$) (0.6) 159 (M-79$^+$) (1.6).

Enolate oxidation of saussurea lactone (3). The enolate oxidation of compound **3** was attempted in the same manner as the enolate oxidation of compound **4**. Compound **3** (114mg, 0.49mmol) was first reacted with 0.36ml (0.54mmol) of LDA and then with oxygen. Dry column (silica gel) chromatography[11] was used to isolate the products eluting with 80/20 hexane/ethyl acetate. 43mg of compound **3** was recovered. 3.1mg (5%) of the decomposition product **(10)** was isolated along with 2.2mg (3%) of 11α-hydroxysaussurea lactone **(8)**, 4.2mg (6%) of 11β-hydroxysaussurea lactone **(7)**, and less than 1mg of 11α-hydroperoxysaussurea lactone **(9)**. The reaction was repeated with 93mg of compound **3** producing 9mg (13%) of compound **10**, 4.0mg(5%) of compound **8**, and 6mg (8%) of compound **7**. The ^{1}H NMR (Figs. 3.23, 3.22, 3.24, and 3.25) IR, and MS data for compounds **7**, **8**, **9**, and **10** are reported in Chapter 3, Part A, Experimental Section.

Allylic oxidation of 11β-hydroxysaussurea lactone (7) to 11β,15-dihydroxysaussurea lacttone (11) and 11β-hydroxy-15-oxosaussurea lactone (12). Selenium dioxide (1.3mg, 0.012mmol) was transferred to a round-bottom flask that contained 15ml of DCM. tert-Butyl hydroperoxide (70% solution,

6.6μl, 0.048mmol) was added to the flask by syringe. The solution was stirred at room temperature for 15 minutes. Then, 6mg (0.024mmol) of compound **7** was added. The reaction was monitored by TLC. After 20 hrs., TLC (silica gel, eluting with 70/30 hexane/ethyl acetate) showed some starting material still present (yellow spot after spraying with $CoCl_2/H_2SO_4$ and applying heat, R_f=0.65), a major product (pink spot, R_f=0.20), and a minor product (grey spot, R_f=0.46). The reaction was stopped, the solution was filtered, and the DCM was evaporated. Preparative TLC was used to isolate 1mg (16%) of compound **12** and 1.3mg (20%) of compound **11**.

11β,15-dihydroxysaussurea lactone (11) IR 3423, 1778, 1638cm^{-1}; ^1H NMR (Fig. 4.4): δ 5.76 (dd,1H,C_1-H,J=11,17Hz), 5.39 (s,1H,C_3-Hb), 5.01 (m,3H,C_2-Ha,b,C_3-Ha,b), 4.61 (dd,1H,C_6-H,J=10,12Hz), 4.10 (br,m,2H,C_{15}-H), 2.33 (d,1H,C_5-H,J=12Hz), 1.47 (s,3H,C_{13}-CH_3), 1.11 (s,3H,C_{14}-CH_3); MS *m/z* (relative intensity) 266 (M^+) (5.7), 251 (M-15^+) (8.5), 240 (M-26^+) (8.1), 238 (M-28^+) (7.7), 201 (M-65^+) (9.3), 91 (100), 43 (59).

11β-hydroxy-15-oxosaussurea lactone (12) IR 3449, 1752, 1691cm^{-1}; ^1H NMR (Fig. 4.5): δ 9.44 (s,1H,C_{15}-H), 6.28 (d,2H,C_3-Ha,b), 5.67 (dd,1H,C_1-H,J=11,17Hz), 4.80 (m,2H,C_2-Ha,b), 4.70 (dd,1H,C_6-H,J=9Hz), 3.03 (d,1H,C_5-H,J=12Hz), 1.47 (s,3H,C_{13}-CH_3), 1.03 (s,3H,C_{14}-CH_3); MS *m/z* (relative intensity) 107 (6.0), 95 (38), 81 (100), 43 (77).

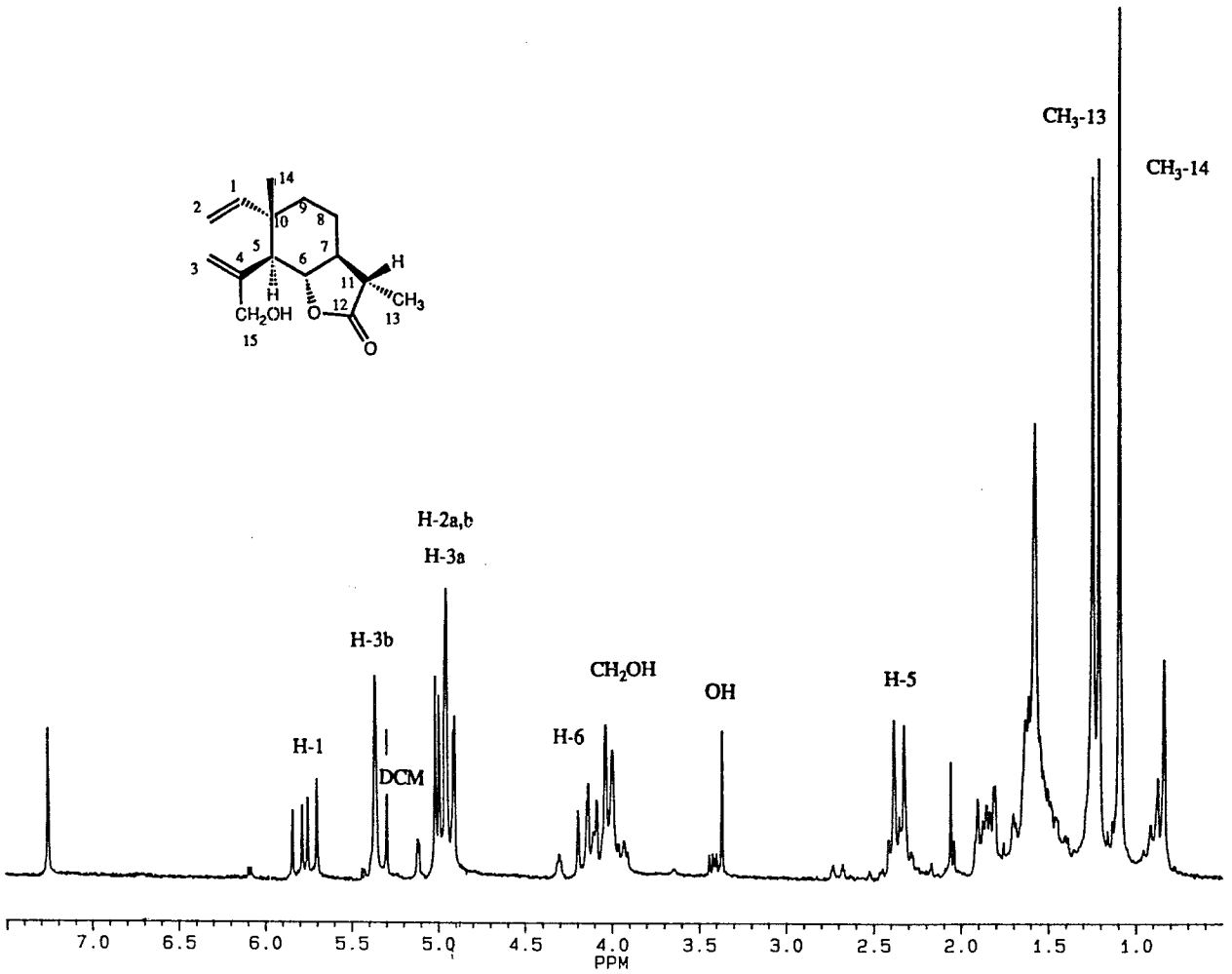


Figure 4.1. ^{1}H NMR spectrum of compound **4** in $CDCl_3$.

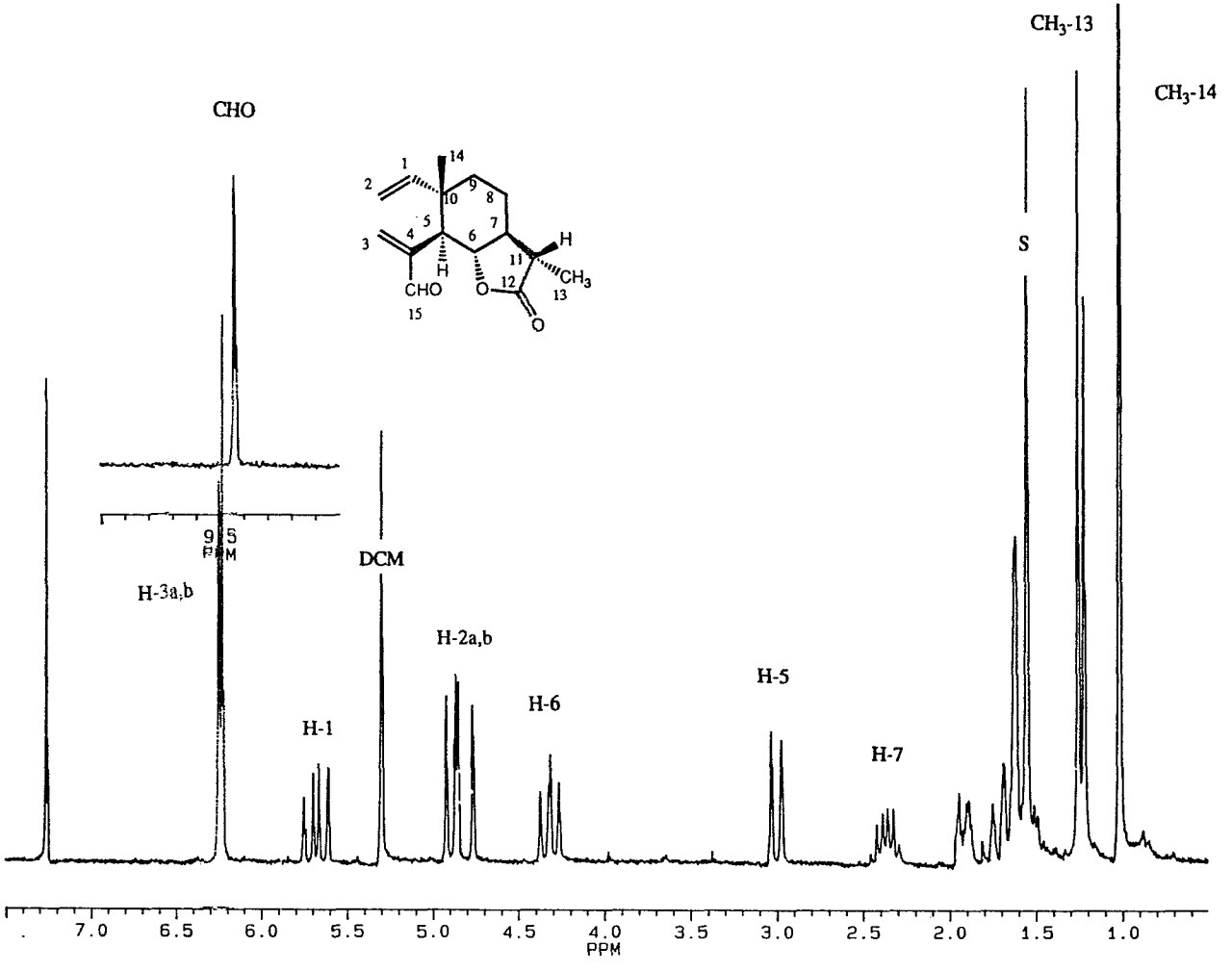


Figure 4.2. ^{1}H NMR spectrum of compound **5** in $CDCl_3$.

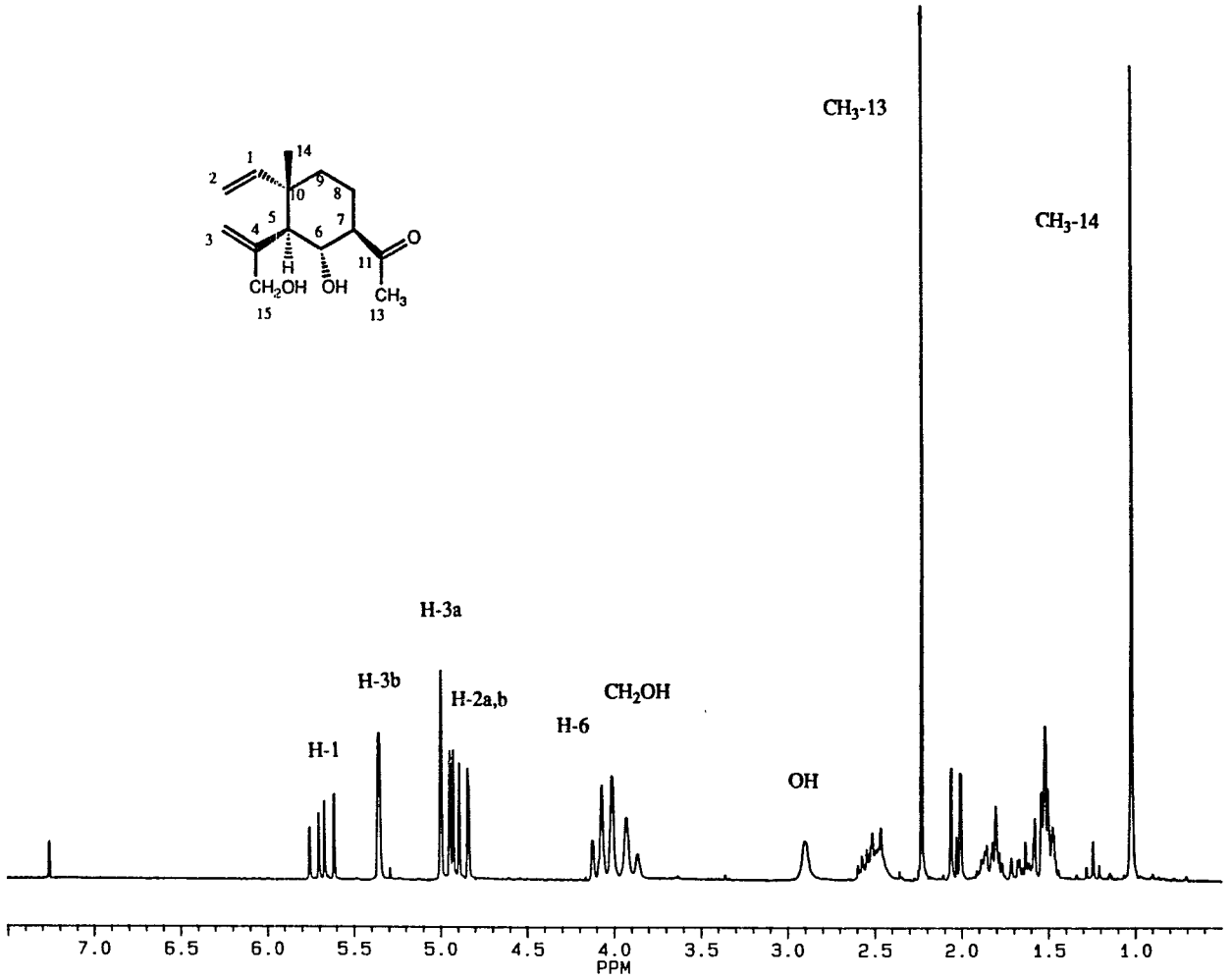


Figure 4.3. ^{1}H NMR spectrum of compound **6** in $CDCl_3$.

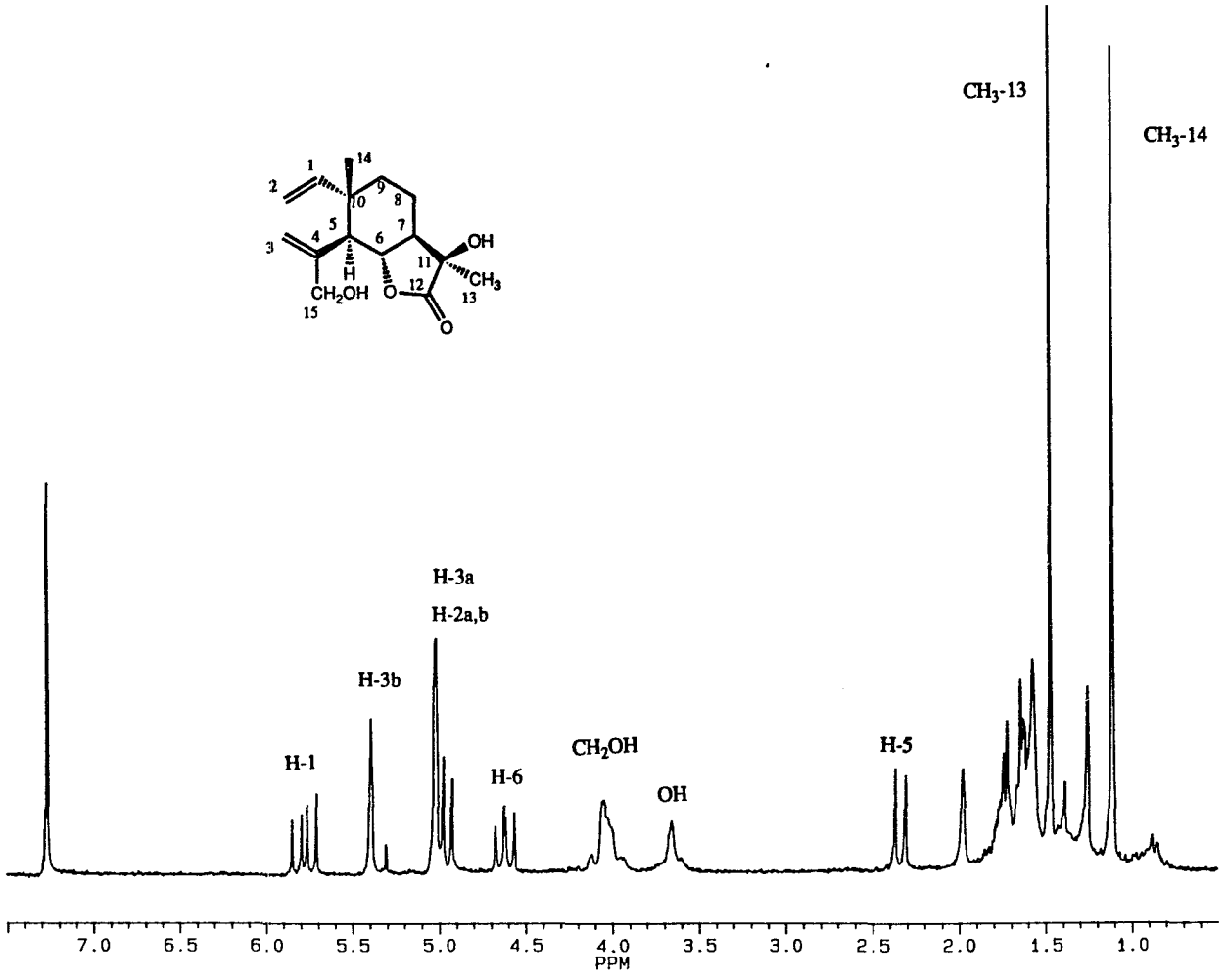


Figure 4.4. ^{1}H NMR spectrum of compound **11** in $CDCl_3$.

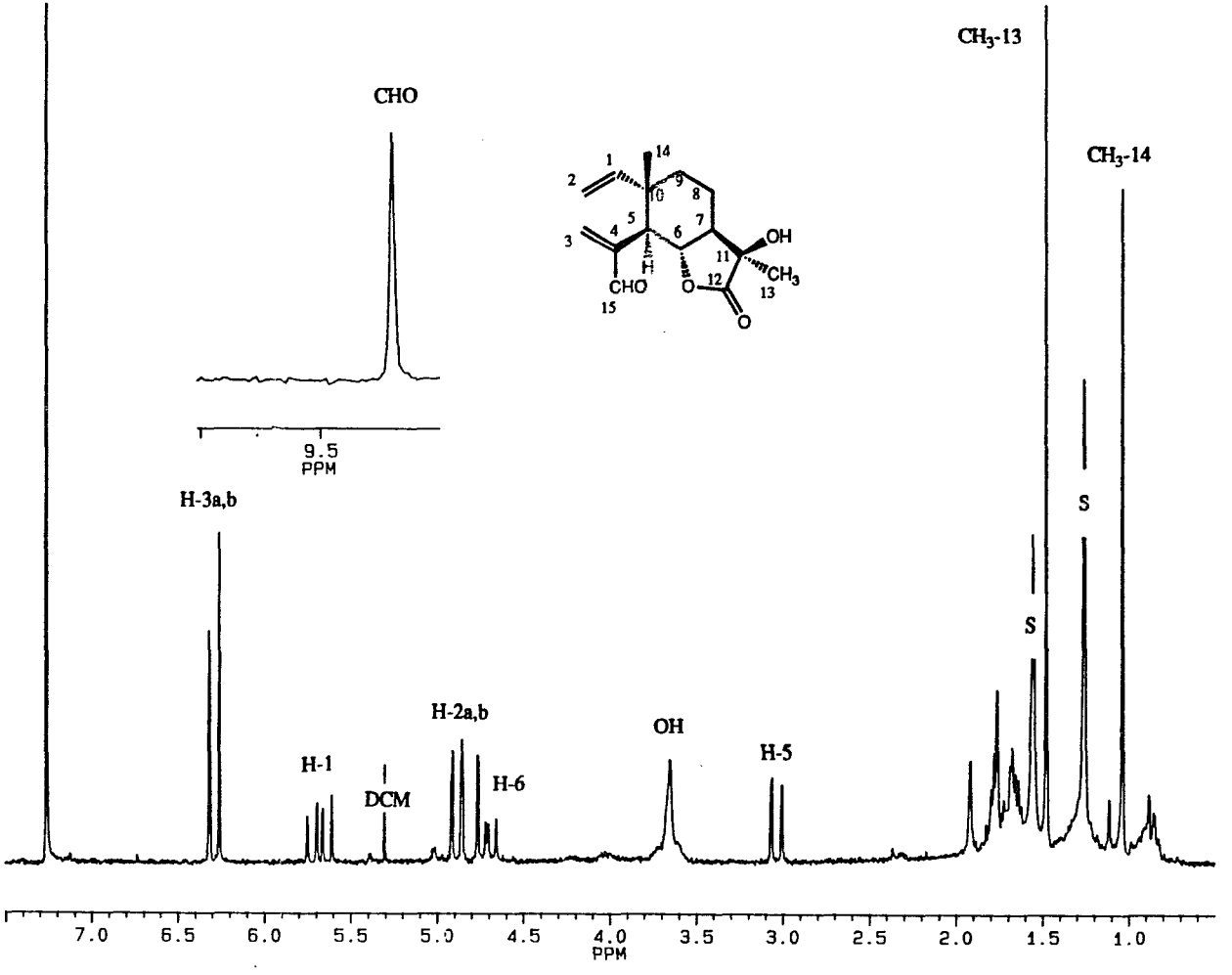


Figure 4.5. ^{1}H NMR spectrum of compound **12** in $CDCl_3$.

References

1. Vargas, D.; Younathan, E.S.; Fischer, N.H., unpublished results.
2. Gonzalez, A.G.; Barrera, J.B.; Garcia, T.Z.; Rosas, F.E. *Phytochemistry* **1984**, *23*, 2071-2.
3. Rao, A.S.; Kelkar, G.R.; Bhattacharyya, S.C. *Tetrahedron* **1960**, *9*, 275-83.
4. Lee, J.Y. Dissertation, Louisiana State University, Baton Rouge, Louisiana , p.107.
5. Rao, A.S.; Sadgopal, A.P.; Bhattacharyya, S.C. *Tetrahedron* **1961**, *13*, 319-23.
6. Umbreit, M.A.; Sharpless, K.B. *J. Amer. Chem. Soc.* **1977**, *99*, 5526-8.
7. Haruna, M.; Ito, K. *J.C.S. Chem. Comm.* **1981**, 483-5.
8. Ando, M.; Tajima, K.; Takase, K. *J. Org. Chem.* **1983**, *48*, 1210-16.
9. Collado, I.G.; Macias, F.A.; Massanet, G.M.; Molinillo, J. M.; R.-Luis, F. J. *Org. Chem.* **1987**, *52*, 3323-6.
10. Pentes, H.; Fischer, N.H.; Macias, F., unpublished results.
11. Loev, B.; Goodmann, M. M. *Chem. and Ind.* **1967**, 2026-32.

Part B. Synthesis of 15-Hydroxydihydrocostunolide From Costunolide

Cope Rearrangements of Elemanolides: Synthesis of 15-Hydroxydihydrocostunolide From Costunolide

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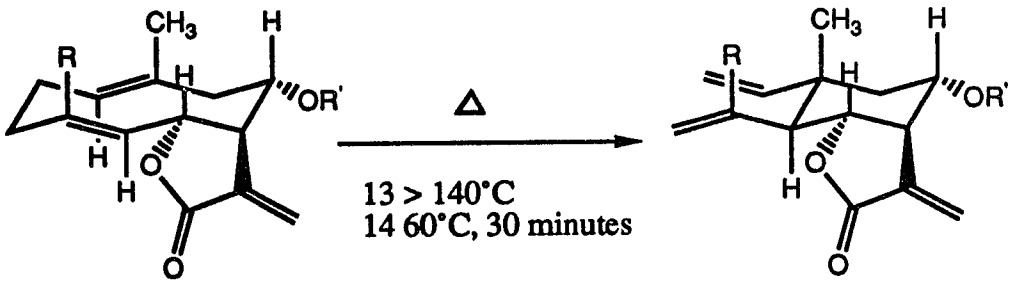
The Cope rearrangement of the C-15-hydroxy- (**4**) and the C-15-oxo- (**5**) elemanolides have been investigated. The Cope rearrangement of compound **4** produces the expected germacranolide product (**17**) which is a natural compound isolated from the roots of *Platycaroha glomerata*. The Cope rearrangement of compound **5** produces a guaianolide type compound (**19**) presumeably through a transannular Michael-type cyclization.

Introduction

The Cope rearrangement of germacranolides to elemanolides is a reversible process which has been used in the total synthesis of sesquiterpene lactones like costunolide.[1] The equilibrium for this reaction usually favors the formation of the thermodynamically more stable isomer; that is the elemanolide. Bohlmann[2] reported that the Cope rearrangement of germacranolides with electron withdrawing substituents at C-4 occur under relatively mild conditions (60°C for thirty minutes, Scheme 4.3). Fischer[3] contends that, based on these results, *in vivo* Cope rearrangements may very well be spontaneous and not enzyme controlled processes.



13 R = CH_2OH
14 R = CHO

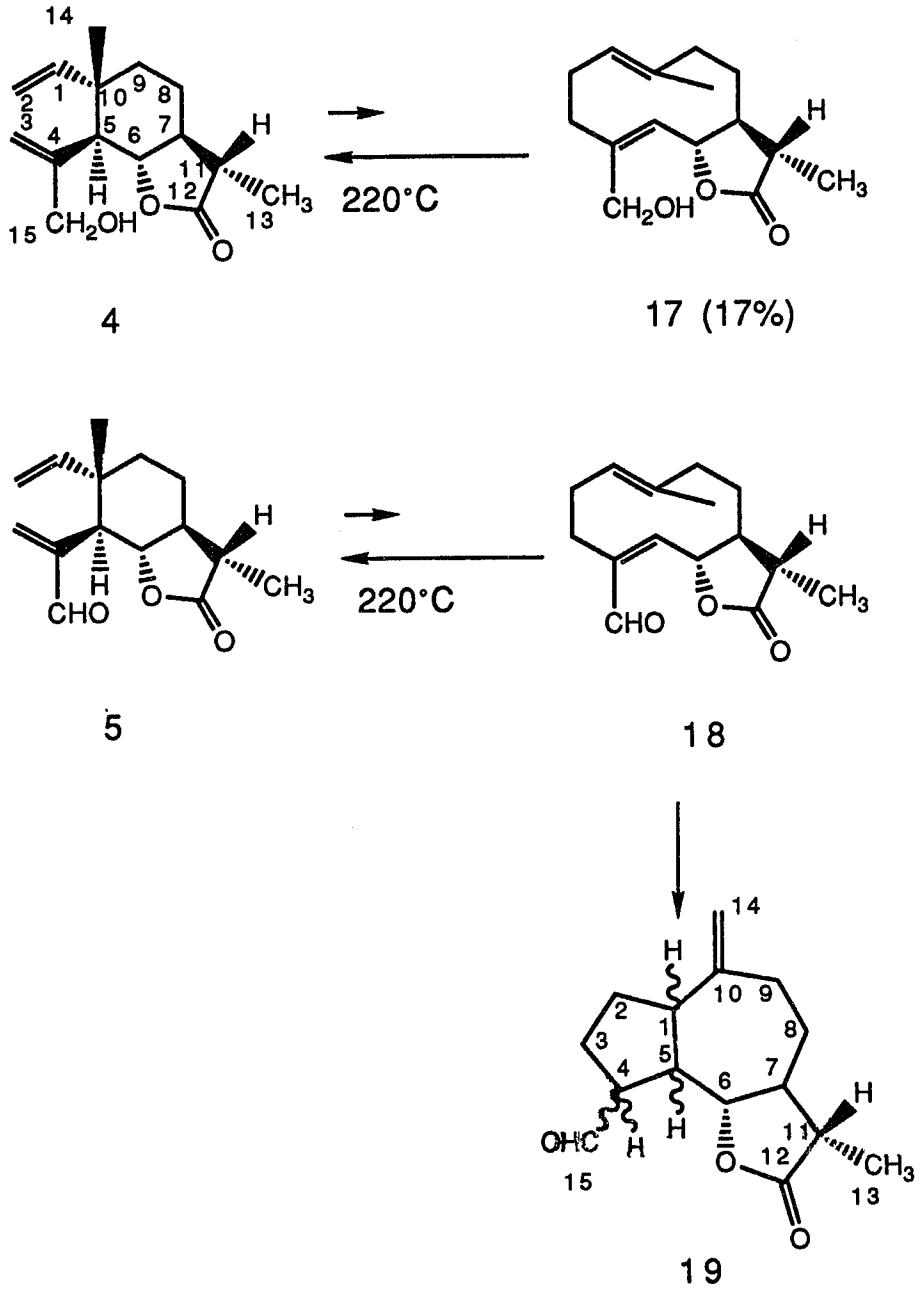
15 R = CH_2OH
16 R = CHO

Scheme 4.3

Results and Discussion

In order to further test this hypothesis, it was attempted to carry out the Cope rearrangement of compounds **4** and **5** at 60°C, neat, for thirty minutes to see whether or not the electron withdrawing group (CHO) at C-4 lowers the activation energy necessary for the Cope rearrangement for the reverse reaction (elemanolide to germacranolide, Scheme 4.4). The ^{1}H NMR spectra of the products were identical to the starting compounds **4** and **5**. Apparently, the electron withdrawing substituent does not lower the activation energy enough to convert the thermodynamically more stable compound **5** to the less stable compound **18** at 60°C.

The Cope rearrangements of compounds **4** and **5** were then carried out at 220°C for three minutes in separate NMR tubes. From the product mixture of the Cope rearrangement of compound **4**, 8mg of recovered **4** and 2mg of 15-hydroxydihydrocostunolide (**17**) were isolated by preparative TLC. The IR spectrum of compound **17** showed an OH absorption at 3600cm^{-1} and a lactone carbonyl absorption at 1768cm^{-1}. The ^{1}H NMR of compound **17** clearly shows the disappearance of the angular methyl group (C-14) signal in compound **4** at 1.1ppm and the appearance of a signal for a methyl group on a double bond at 1.4ppm in compound **17**. The ^{1}H NMR spectrum of compound **17** also shows the presence of only 2 olefinic protons (H-1 and H-5) whereas the ^{1}H NMR spectrum of compound **4** shows the presence of 5 olefinic protons. The ^{1}H NMR spectrum of compound **17** in C_6D_6 was identical to the natural compound isolated by Bohlmann and Zdero from *Platycaroha glomerata*.[4] Thus, 15-hydroxydihydrocostunolide (**17**), has been synthesized in four steps from costunolide with an overall yield of 9%.


14
1
2
3
4
5
6
7
8
9
10
11
12
13
15
H
H
O
O
CH3
CH2OH
220°C
CH2OH
H
CH3
O
O
4
17 (17%)
H
H
CHO
O
O
CH3
220°C
CHO
H
CH3
O
O
5
18
14
H
H
OHC
H
15
O
O
CH3
13
19

Scheme 4.4

Column chromatography was used to separate an unexpected guaianolide type compound (**19**) from the Cope rearrangement product mixture of compound **5**. The IR spectrum of compound **19** showed a lactone carbonyl absorption at 1776cm^{-1}, a non-conjugated aldehyde absorption at 1722cm^{-1}, and a carbon-carbon double bond absorption at 1638cm^{-1}. The ^{1}H NMR spectrum of compound **19** shows the disappearance of the methyl singlet for the angular methyl group (C-14) in compound **5** and the appearance of two exocyclic methylene signals at 4.9 and 5.0ppm. The ^{13}C NMR spectrum of compound **19** shows two carbonyl resonances (202 and 178ppm) and two carbon-carbon double-bond resonances (149 and 112ppm). The DEPT 135 experiment clearly identifies the resonance at 112ppm as an exocyclic methylene signal (Fig. 4.9). The ^{13}C NMR assignments for compound **19** are shown in Table 4.1. This data lead us to propose the guaianolide type structure for compound **19** which could result from a transannular Michael-type cyclization of the expected Cope product (**18**). The absolute configuration at C-1, C-4, and C-5 are not known; however, H-1 and H-5 are probably both alpha oriented if the mechanism for formation of this product is concerted and if **18** exists in a frozen solid-state conformation similar to both costunolide[5] and tamaulipin A[6] [$_{1}$D^{14},^{15}D$_{5}$][7] in which both C-14 and C-15 are beta oriented (Scheme 4.5).

Experimental Section

^{1}H NMR spectra were recorded on a Bruker-AC200 spectrometer in $CDCl_3$ using Me_4Si as an internal standard. Mass spectra were recorded on a HP5985 spectrometer. Infrared spectra were recorded on a Perkin-Elmer 1760x Spectrometer in film on NaCl plates. Column chromatographic separations were made on silica gel (60-200M, J. T. Baker Co.). Preparative TLC separations were made on SIL G-100 (or 50) Sybron/Brinkmann plates.

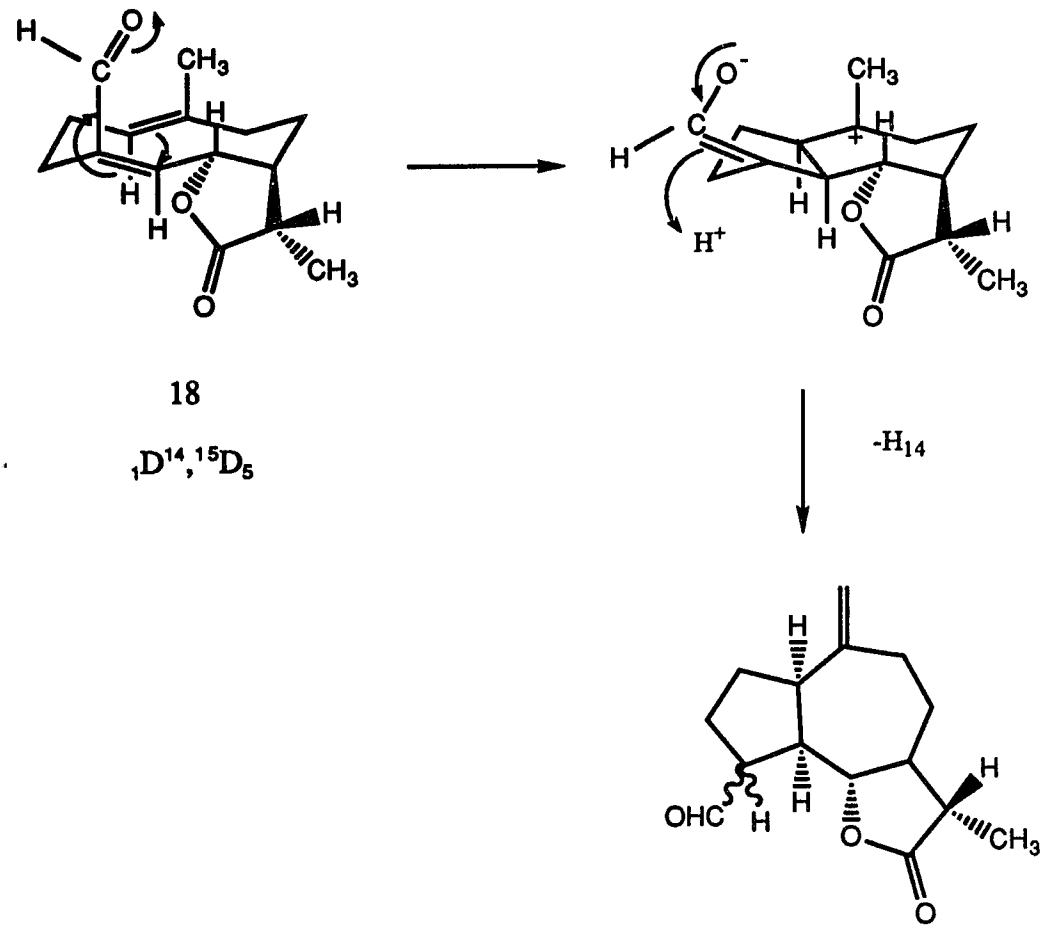
Table 4.1. ^{13}C NMR assignments[a] for compound **19**

Carbon atom	ppm
1	56.7[b]
2	36.7[c]
3	32.5[c]
4	52.3[b]
5	46.8[b]
6	84.5
7	46.5[b]
8	30.4[c]
9	26.7[c]
10	149.0
11	42.6[b]
12	177.8
13	13.0
14	112.7
15	202.4

a ^{13}C NMR recorded in $CDCl_3$ at 200 MHz

b = assignments are interchangeable

c = assignments are interchangeable



18

$_1D^{14},^{15}D_5$

Scheme 4.5

Compounds **4** and **5** can be synthesized from costunolide in three steps with an overall yield of 52% and 7% respectively.[8]

Attempted Cope rearrangement of compounds 4 and 5 at 60°C. Compound **4** (12mg) and compound **5** (35mg) were heated at 60°C in a mineral oil bath in separate NMR tubes (neat) for thirty minutes. The ^1H NMR spectra of these samples after thirty minutes showed no discernable difference from the starting compounds.

Attempted Cope rearrangement of compounds 4 and 5 at 220°C. Compound **4** (12mg) and compound **5** (35mg) were heated at ~220°C in a mineral oil bath in separate NMR tubes for three minutes. The product mixture from the Cope rearrangement of compound **4** was separated by preparative TLC eluting with 60/40 hexane/ethyl acetate. Two products were distinguishable by TLC: a pink spot visible after spraying with $CoCl_2/H_2SO_4$ and applying heat ($R_f = 0.45$) and a grey spot ($R_f = 0.38$). ^1H NMR analysis showed the less polar compound to be recovered compound **4** (8mg). The more polar compound was determined to be identical to the natural sesquiterpene lactone 15-hydroxydihydrocostunolide (**19**) (2mg, 17%). IR 3600, 1768cm^{-1}; ^1H NMR (Fig. 4.6): δ 4.88 (dd,1H,C_1-H), 4.75 (m,2H,C_5-H,C_6-H), 4.30 (d,1H,C_{15}-H,J=13Hz), 4.00 (d,1H,C_{15}-H,J=13Hz), 1.36 (s,3H,C_{14}-CH_3), 1.26 (d,3H,C_{13}-CH_3,J=7Hz); ^1H NMR in C_6D_6 (Fig. 4.7): δ 4.55 (dd,1H,C_1-H,J=8Hz), 4.27 (m,2H,C_5-H,C_6-H), 3.90 (d,1H,C_{15}-H,J=13Hz), 3.64 (d,1H,C_{15}-H,J=13Hz), 1.04 (s,3H,C_{14}-CH_3), 1.03 (d,3H,C_{13}-CH_3,J=7Hz); MS *m/z* (relative intensity) 250 (M^+) (0.1), 232 (M-18$^+$) (2.2), 219 (M-31$^+$) (9.1), 55 (100).

The Cope rearrangement product of compound **5** was purified by dry column (silica gel) chromatography[9] eluting with dichloromethane. A guaianolide type sesquiterpene lactone (**19**) (10mg) was isolated and analyzed. IR 1776, 1722,

1638cm^{-1}; ^{1}H NMR (Fig. 4.8): δ 9.78 (d,1H,C_{15}-CHO,J=2Hz), 4.93 (s,1H,C_{14}-H), 4.89 (s,1H,C_{14}-H), 3.86 (dd,1H,C_6-H,J=10Hz), 1.21 (d,3H,C_{13}-CH_3,J=7Hz); MS *m/z* (relative intensity) 248 (M^+) (1.9), 219 (M-29$^+$) (1.7), 202 (M-46$^+$) (5.3), 91 (100).

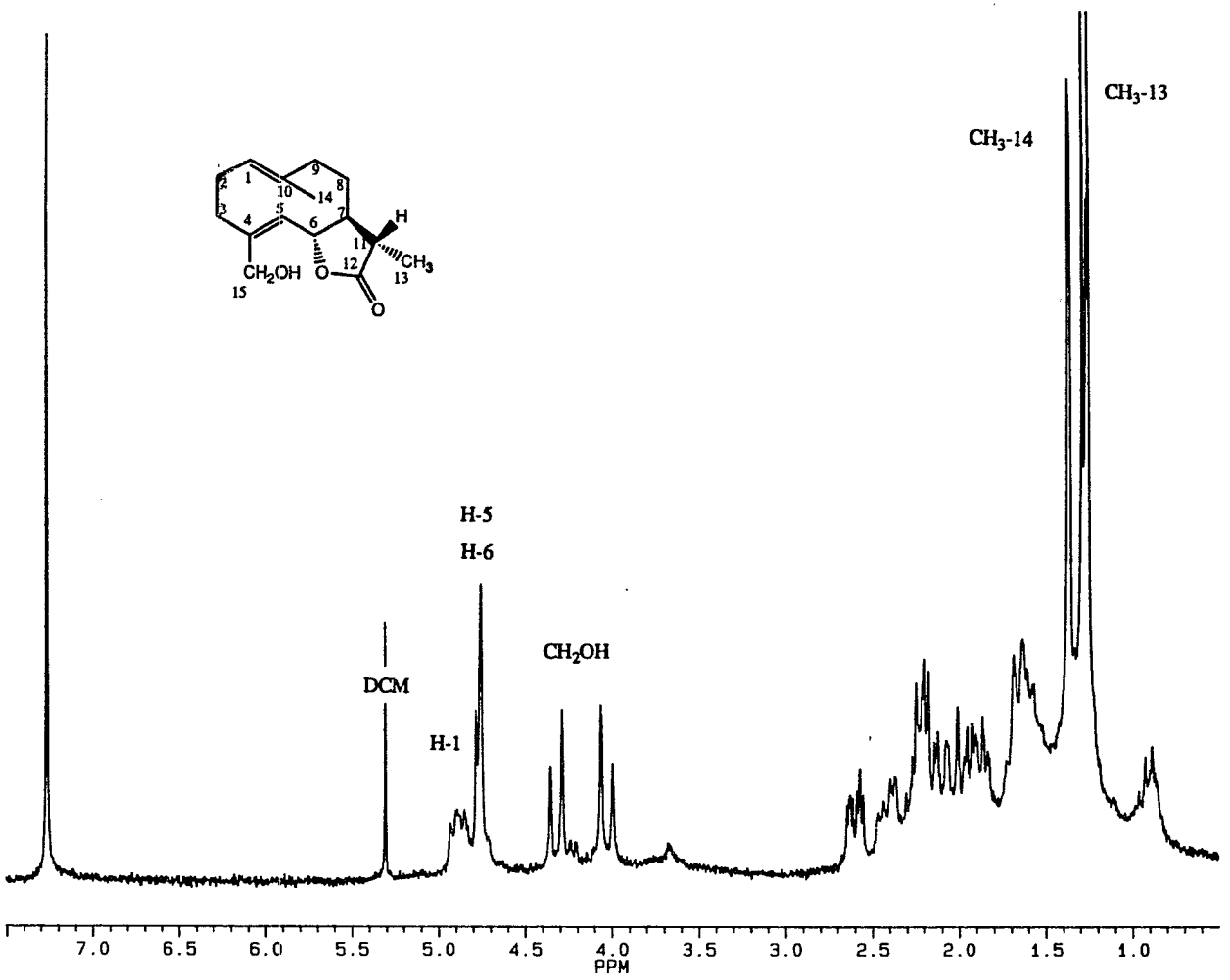


Figure 4.6. ^{1}H NMR spectrum of compound **17** in $CDCl_3$.

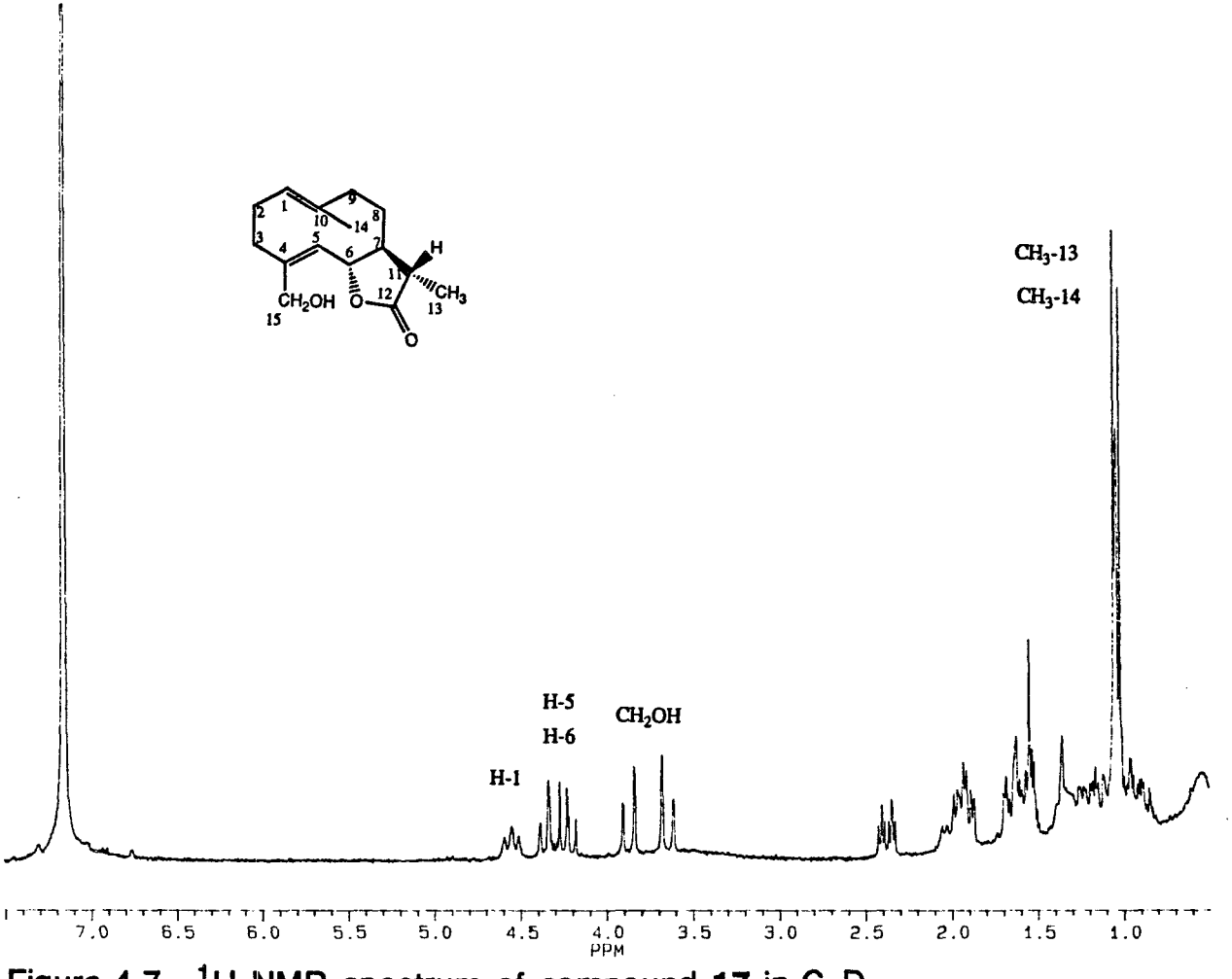


Figure 4.7. ^{1}H NMR spectrum of compound **17** in C_6D_6.

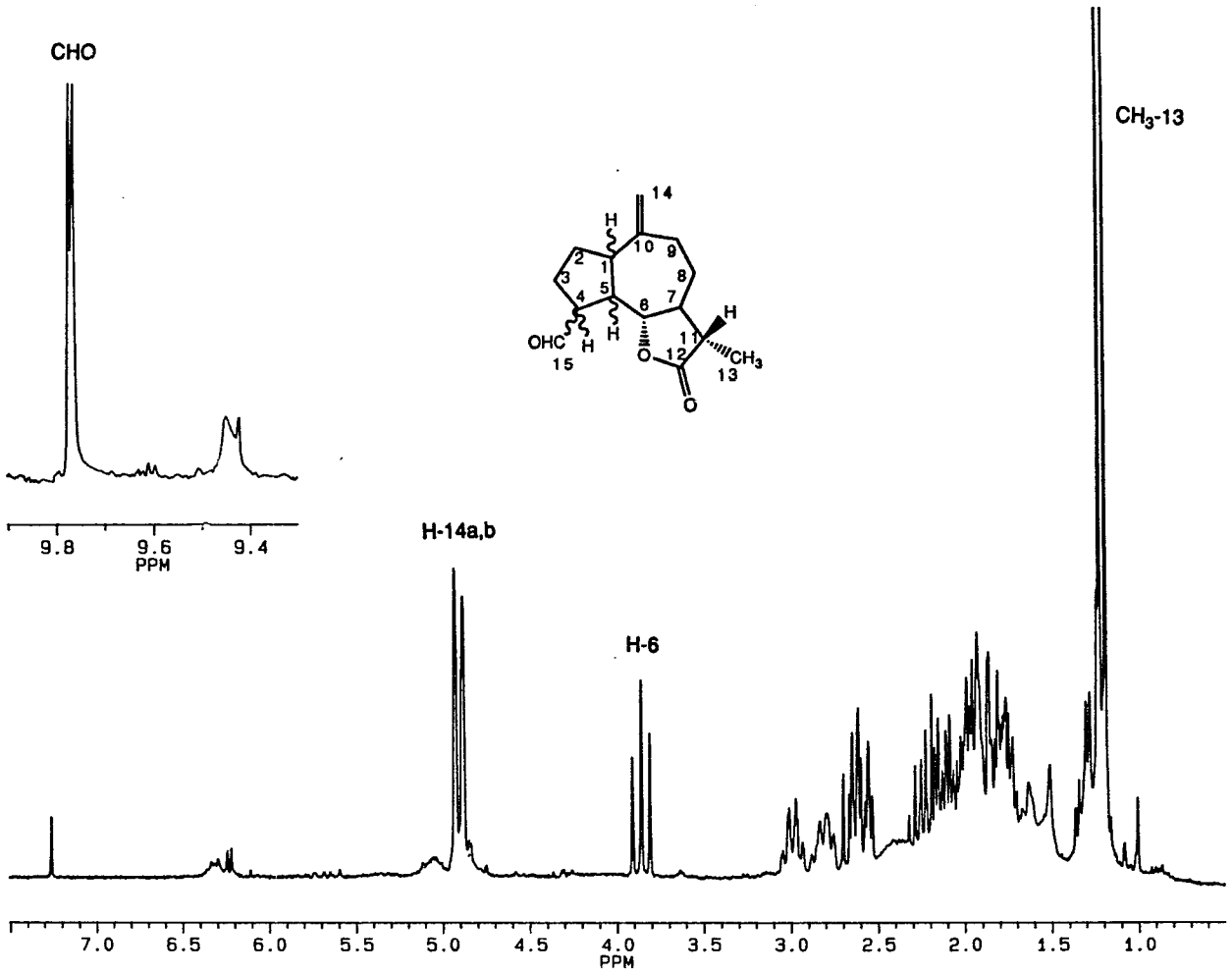


Figure 4.8. ^{1}H NMR spectrum of compound **19** in $CDCl_3$.

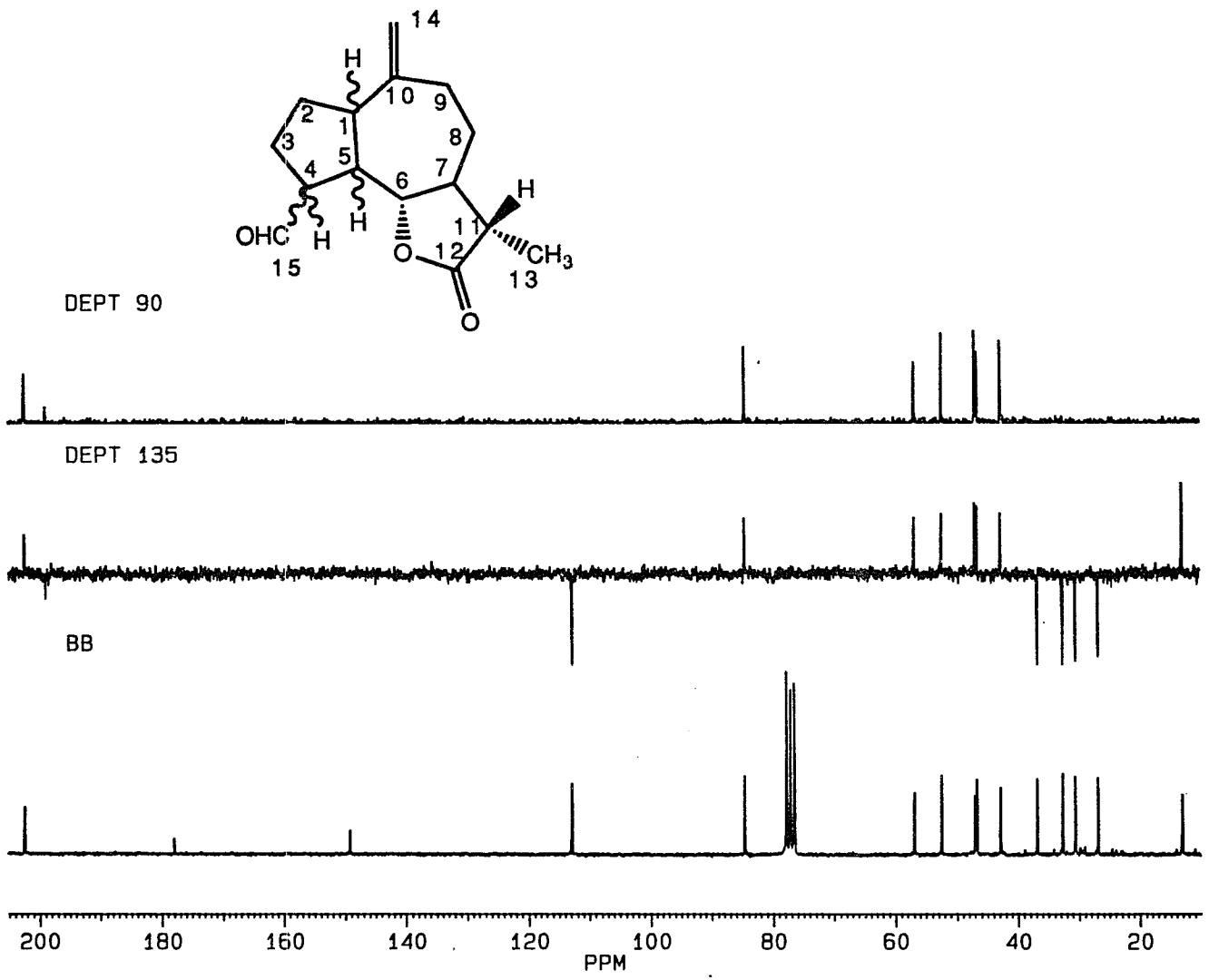


Figure 4.9. DEPT 90, 135, and BB ^{13}C NMR spectrum of **19** in $CDCl_3$.

References

1. Grieco, P.A.; Nishizawa, M. *J. Org. Chem.* **1977**, *42*, 1717-20.
2. Bohlmann, F.; Zdero, C. *Phytochemistry* **1979**, *18*, 95-8.
3. Fischer, N.H. Phytochemical Society Reviews **1979**. "Sesquiterpene lactones. Biogenesis and Biomimetic Transformations."
4. Bohlmann, F.; Zdero, C. *Phytochemistry* **1977**, *16*, 1832-4.
5. Bovill, M.J.; Cox, P.J.; Cradwick, P.D.; Guy, M.H.P.; Sim, G.A.; White, D.N.J. *Acta. Cryst.*, Sect. B **1976**, *32*, 3203-9.
6. Witt, M.E.; Watkins, S.F. *J. Chem. Soc. Perkin Trans.* **1978**, *2*, 204-8.
7. Symbolism proposed by Samek and Harmatha. Samek,Z.; Harmatha, J. *Collect. Czech. Chem. Commun.* **1978**, *43*, 2779-99.
8. Pentes, H.; Fischer, N.H. unpublished results.
9. Loev, B.; Goodmann, M. M. *Chem. and Ind.* **1967**, 2026-32.

Part C. Peroxydihydroparthenolide: X-ray Crystal Structure and Chemical Conversion to Deoxy- and Anhydro-derivatives.

Peroxydihydroparthenolide: X-ray Crystal Structure and Chemical Conversion to Deoxy- and Anhydro-derivatives.

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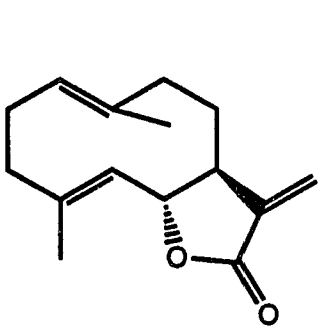
The X-ray crystal structure of peroxydihydroparthenolide, a hydroperoxy-sesquiterpene lactone, has been determined. Peroxydihydroparthenolide has also been converted to its deoxy- and anhydro-derivatives, which were characterized by ^{1}H and ^{13}C NMR, FTIR, and mass spectral data.

Introduction

Naturally occurring hydroperoxy-sesquiterpene lactones have been isolated from the leaves of *Magnolia grandiflora* L.[1] and *Liriodendron tulipifera* L.[2,3] Two of these compounds, peroxycostunolide (**21**) and peroxyparthenolide (**23**) were determined to be cytotoxic.[1] The biosynthesis of these hydroperoxy-sesquiterpene lactones is unknown, however, it is believed to involve chlorophyll mediated singlet oxygen oxidation of the 1,10-double bond of the corresponding germacrolides.[2,5] Evidence for these transformations is provided by previous *in vitro* singlet oxygen photooxygenations of costunolide (**20**), parthenolide (**22**), and dihydroparthenolide (**24**) to compounds **21**, **23**, and peroxydihydroparthenolide (**25**) (Scheme 4.6)[4].



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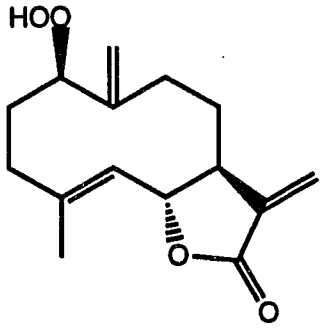

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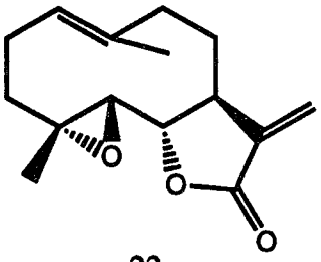

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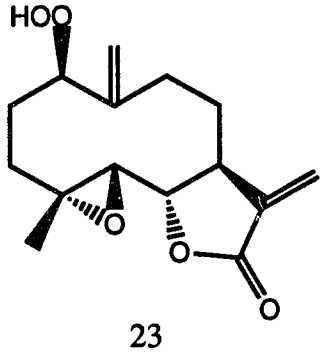

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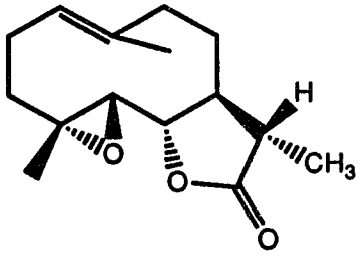

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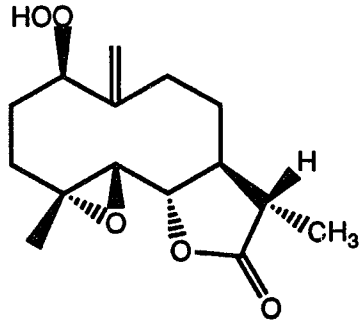


25

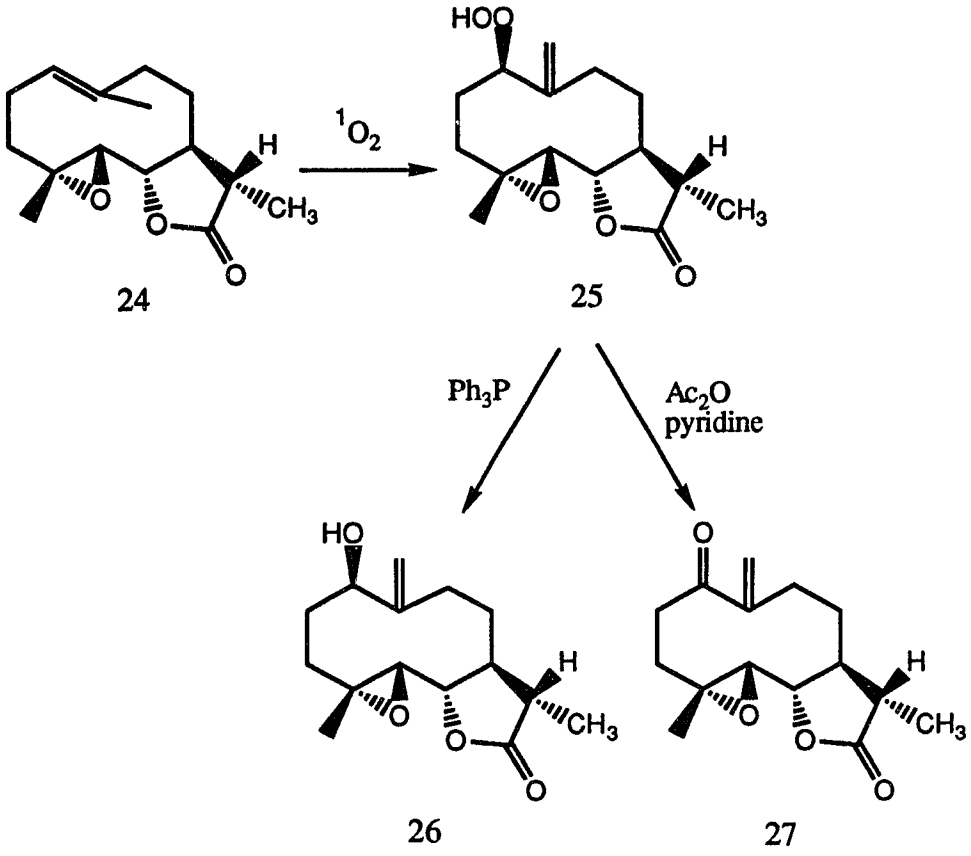
Scheme 4.6

Results and Discussion

The singlet oxygen reaction of dihydroparthenolide (**24**) to peroxydihydroparthenolide (**25**) was carried out in dichloromethane (DCM) using methylene blue as the photosensitizing agent[4] (Scheme 4.7). Oxygen was bubbled through the solution which was irradiated with visible light. The molecular structure of **25** was confirmed by single crystal X-ray diffraction which will be discussed later. Peroxydihydroparthenolide (**25**) was converted to its deoxy-derivative (**26**) by reduction with triphenylphosphine in DCM. Peroxydihydroparthenolide (**25**) was also converted to its anhydro-derivative (**27**) by reduction with acetic anhydride in pyridine.

The ^{1}H NMR data and assignments for compounds **25**,**26**, and **27** are compiled in Table 4.2. H-1 and H-14a,b shift upfield (-0.14, -0.25, and -0.09ppm, respectively) on reducing the hydroperoxide (**25**) to the alcohol (**26**) because the -OOH substituent is a stronger deshielding substituent than the -OH group is. H-14a,b shift downfield (0.69 and 0.55ppm, respectively) on reducing the hydroperoxide (**25**) to the ketone (**27**) because of conjugation. The ^{1}H NMR data for (**25**) in d_6-acetone is in good agreement to that previously reported by El-Feraly et al. [4]

The ^{13}C NMR data and assignments for compounds **25**, **26**, and **27** are compiled in Table 4.3. The ^{13}C NMR assignments were made by comparison with literature data (**25** in pyr-d_5).[4] The downfield β shift of C-10 (5.1ppm) and the upfield γ shift of C-14 (2.9ppm) which occurs for the allylic hydroperoxide (**25**) relative to the alcohol (**26**) are consistent with the ^{13}C NMR data of similar compounds.[4] These shifts occur when an oxygen atom is at an anti-clinal position with respect to a double-bond.[6] The effective electronegativity of the oxygen atom



Scheme 4.7

Table 4.2. ^{1}H NMR data for compounds **25**,**26**, and **27**[a]

Compound	H-1	H-2	H-5	H-6	H-14a,b	CH_3-13	CH_3-15	miscellaneous
25	4.33 dd(4,11)		2.78 d(9)	3.76 t(9)	5.21 s, 5.46 s	1.29 d(6)	1.45 s	7.83 s (OOH)
25[b]	4.27 dd(4,11)		2.81 d(9)	3.86 t(9)	5.22 s, 5.38 s	1.20 d(7)	1.40 s	
26	4.19 dd(5,11)		2.77 d(9)	3.76 t(9)	4.96 s, 5.37 s	1.28 d(7)	1.49 s	
27		2.84 m	2.63 d(9)	3.61 t(9)	5.90 s, 6.01 s	1.28 d(7)	1.36 s	

[a]Spectra were recorded in $CDCl_3$ at 200 MHz with Me_4Si as internal standard. Chemical shifts are in ppm; coupling constants, J, are in Herz given in parentheses; and multiplicities are designated by the following symbols: s=singlet, d=doublet, t=triplet, m=multiplet.

[b] in d_6-acetone.

Table 4.3. ^{13}C NMR assignments for compounds **25**,**26**, and **27**.[a]

Carbon atom	**25**	**26**	**27**
1	91.6	79.9[c]	202.8
2	25.2[b]	24.3[b]	35.5[b]
3	26.4[b]	29.6[b]	31.8[b]
4	60.2	60.2	59.0
5	63.6	63.7	65.5
6	79.8	79.0[c]	81.0
7	47.1	46.2	46.8
8	25.2[b]	24.8[b]	27.3[b]
9	33.9[b]	33.7[b]	34.9[b]
10	143.5	148.6	149.3
11	41.9	42.0	42.4
12	177.7	177.8	177.4
13	12.9	13.0	12.8
14	118.7	115.8	126.5
15	18.2	18.4	17.4

[a] Spectra were determined in $CDCl_3$ at 200 MHz with Me_4Si as internal standard. Chemical shifts are in ppm.

[b] assignments are interchangeable

[c] assignments are interchangeable

is enhanced by a through-space interaction between the π-type n-orbital of the oxygen atom and the vacant pπ*-orbital. This increases the hyperconjugative through-bond interaction between the C-O σ-orbital and the pπ-orbital. The resulting delocalization of the π-orbital electrons results in a downfield β shift and an upfield γ shift for carbon resonances. This effect appears to be larger for the hydroperoxy substituent than the hydroxy substituent.[4] When the oxygen atom is syn-periplanar with respect to the carbon-carbon double bond, these carbon resonance shifts should not occur, because the C-O σ-orbital and the pπ-orbital are essentially perpindicular to one another. The X-ray crystal structure of **25** (Figure 4.17) clearly demonstrates, at least in the solid state, the anti-clinal position of oxygen O4 relative to the C10-C14 double bond.

The presence of an OH group in both compounds **25** and **26** was confirmed by an infrared absorption near 3400cm^{-1}. The infrared spectrum of compounds **25** and **26** also shows a lactonic carbonyl absorption near 1770cm^{-1}. The infrared spectrum of **27** exhibits an additional carbonyl absorption at 1665cm^{-1} due to a conjugated carbonyl group.

The mass spectral pattern of **26** exhibits a peak at 248 (M-18^{+}) indicative of the loss of water. The mass spectral pattern of **27** exhibits a peak at 222 (M-42^{+}[C_2H_2O]) which is typical for α,β-unstaurated ketones.[7]

Experimental Section

^{1}H and ^{13}C NMR spectra were recorded on a Bruker-AC200 spectrometer in $CDCl_3$ using $SiMe_4$ as an internal standard. Mass spectra were recorded on a HP5985 spectrometer. Infrared spectra were recorded either on a Perkin-Elmer 257 or 1760x spectrometer in film on NaCl plates.

X-ray intensity data were collected by ω-2θ scans on an Enraf-Nonius

diffractometer equipped with CuKα radiation (λ = 1.54184Å) and a graphite monochromater. Full sphere of data were measured within $2° < \theta < 75°$. The structure was solved by direct methods and refined by full-matrix least squares using the Enraf-Nonius SDP. Nonhydrogen atoms were refined anisotropically, and hydrogen atoms were refined isotropically.

Peroxydihydroparthenolide (25). A 300mg sample of dihydroparthenolide[8] (**24**) and 10mg of methylene blue were dissolved in 30ml of DCM in a round-bottom flask immersed in an ice/water cooled Dewar flask. Oxygen (industrial grade, Liquid Carbonic) was gently bubbled through the solution. The solution was irradiated with visible light from a tungsten filament lamp placed 15cm above the neck of the flask. The reaction temperature was maintained between 15-25°C. The reaction was stopped after 4 hours. The solvent was evaporated and vacuum liquid chromatography (VLC, MN-Kiesel gel G silica gel) was used to separate unreacted starting material **24** from compound **25** and methylene blue. Compound **24** was eluted from the VLC column with DCM, **25** eluted with ethyl acetate, and methylene blue remained adsorbed to the top of the silica gel column. Evaporation of the ethyl acetate fraction yielded 183mg (54%) of a white crystalline compound (**25**). The spectral and physical data of **25** is identical to that reported previously.[4] Experimental melting point 112-114°C (lit, m.p. 112°C[4]); IR 3370, 1770cm^{-1}; ^{1}H NMR (Table 4.2, Fig. 4.10, and Fig. 4.11); ^{13}C NMR (Table 4.3, Fig. 4.14); MS *m/z* (relative intensity) 282 (M^+) (0.04), 257 ($M-25^+$) (0.6). The molecular structure of peroxydihydroparthenolide (**25**) is shown in Figure 4.17. Crystal data for **25**: $C_{15}H_{22}O_5$, MW = 282.3, triclinic space group P1, a = 5.7473(3), b = 7.6109(7), c = 9.0705(9) Å, α = 70.190(8), β = 85.259(6), γ = 78.188(6)°, Z = 1, Dc = 1.283gcm^{-3}, R = 0.039 for 2810 observed data.

Deoxyperoxydihydroparthenolide (26). A DCM solution of Ph_3P (125mg, 0.48mmols) was slowly added to a magnetically stirred DCM solution of compound **25** (135mg, 0.48mmols). The solution was stirred at room temperature for 15 minutes. Thin-layer chromatography (TLC) showed all of **25** had reacted. Compound **26** (113mg, 89%) was isolated as a white solid by silica gel vacuum liquid chromatography (VLC) eluting with DCM. IR 3432, 1770cm^{-1}; ^1H NMR (Table 4.2, Fig. 4.12); ^{13}C NMR (Table 4.3, Fig. 4.15); MS *m/z* (relative intensity) 266 (M^+) (0.6), 248 ($M-18^+$) (0.4), 237 ($M-29^+$) (0.9), 223 ($M-43^+$) (0.4), 210 ($M-56^+$) (4.2), 207 ($M-59^+$) (42).

Anhydroperoxydihydroparthenolde (27). Compound **25** (43mg, 0.15mmoles) was dissolved in 2ml of pyridine and 3ml of acetic anhydride. The solution was stirred at room temperature for 1 hour and quenched by the addition of 5 grams of ice. The aqueous solution was extracted with DCM (5x10ml). The combined DCM extracts were washed with 5% $NaHCO_3$ (10ml), distilled water (10ml), 0.1N HCl (10ml), and distilled water (10ml). The DCM solution was dried over anhydrous Na_2SO_4, filtered, and the solvent evaporated, yielding a powdery material. Crystallization from diethyl ether yielded 15mg (37%) of **27**. IR 1778, 1665cm^{-1}; ^1H NMR (Table 4.2, Fig. 4.13); ^{13}C NMR (Table 4.3, Fig. 4.16); MS *m/z* (relative intensity) 264 (M^+) (0.07), 249 ($M-15^+$) (2.0), 235 ($M-29^+$) (3.5), 233 ($M-31^+$) (4.3), 231 ($M-33^+$) (1.8), 222 ($M-42^+$) (0.7), 221 ($M-43^+$) (6.5), 207 ($M-57^+$) (23.4).

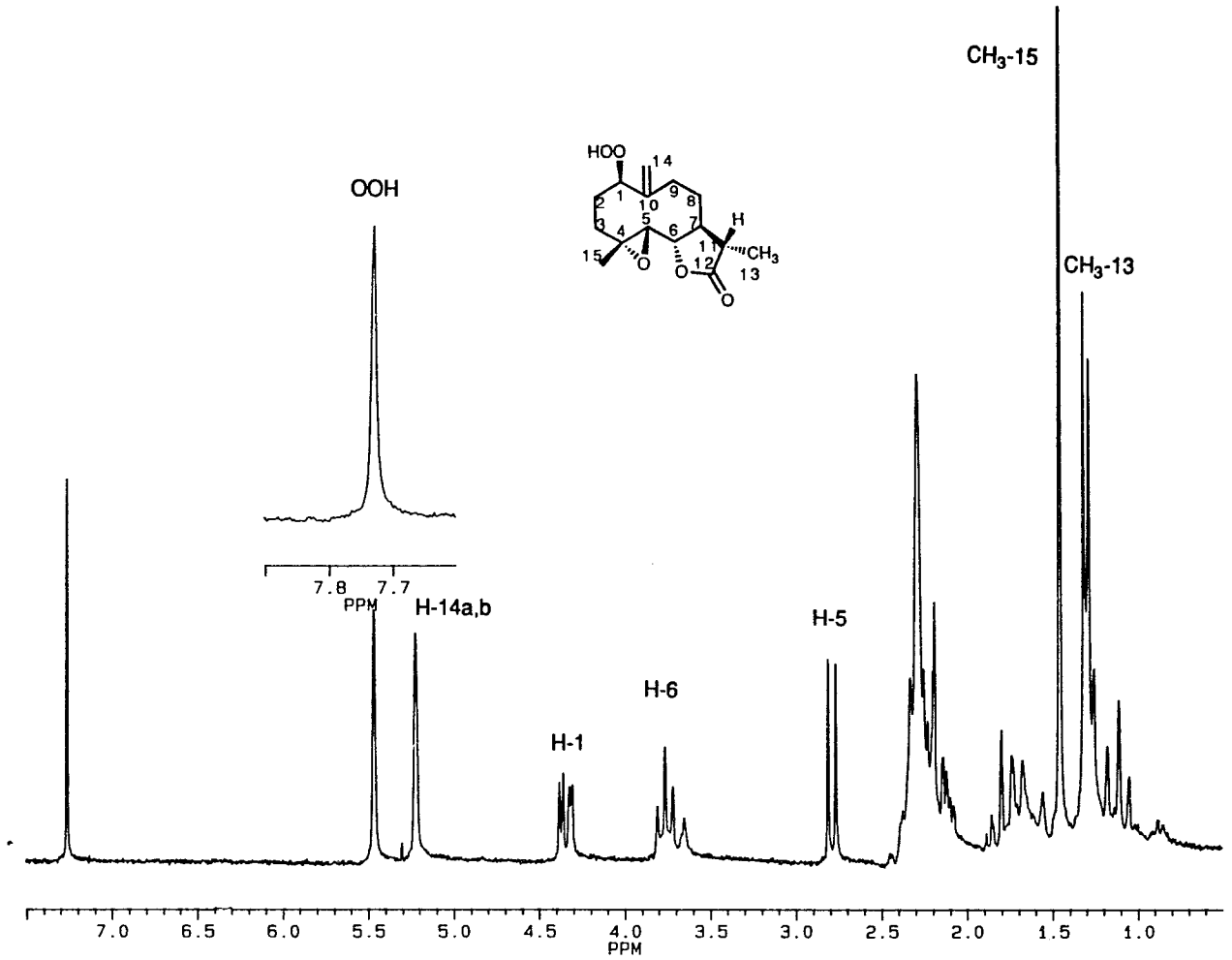


Figure 4.10. ^{1}H NMR spectrum of compound 25 in $CDCl_3$.

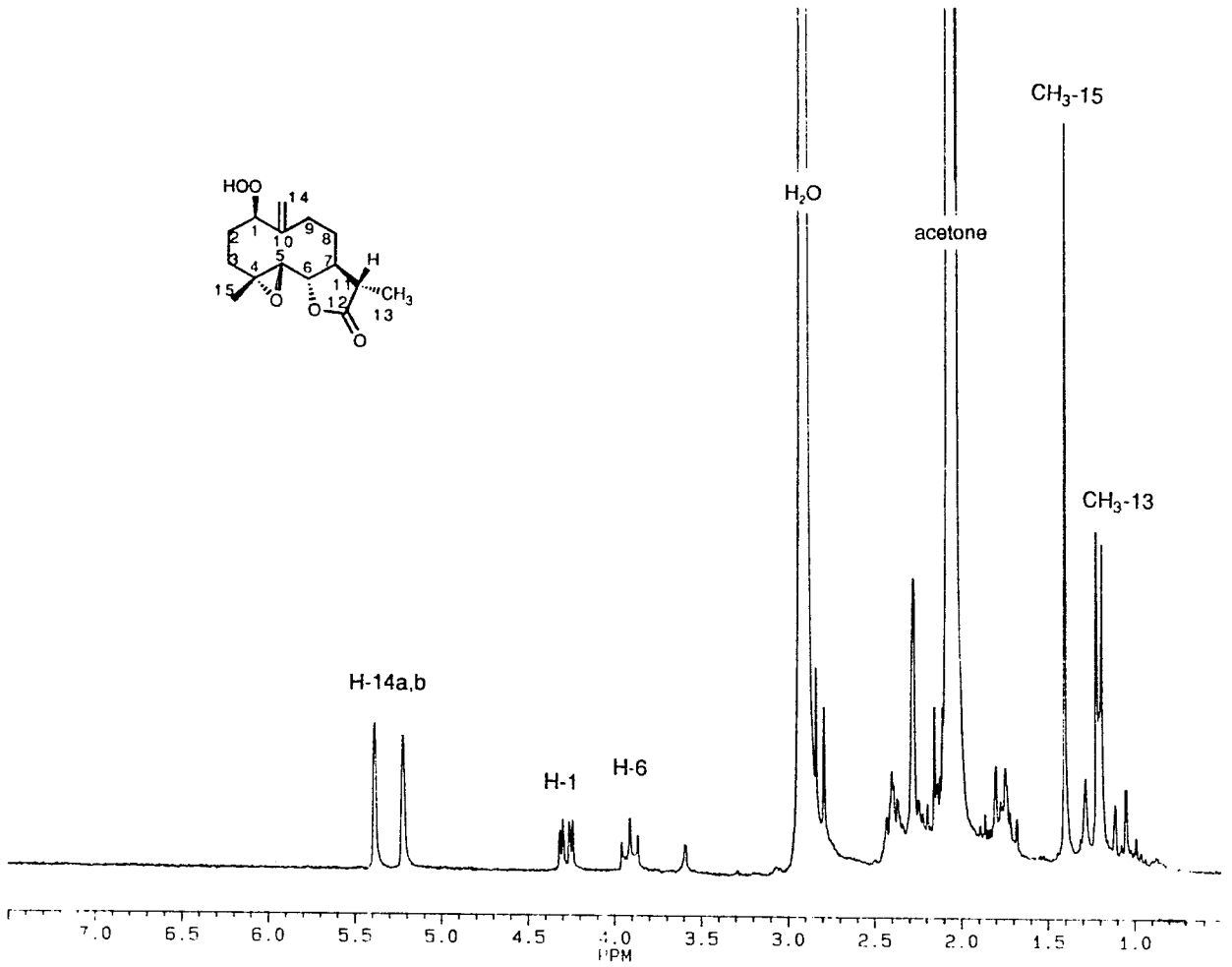


Figure 4.11. ^{1}H NMR spectrum of compound 25 in d_6-acetone.

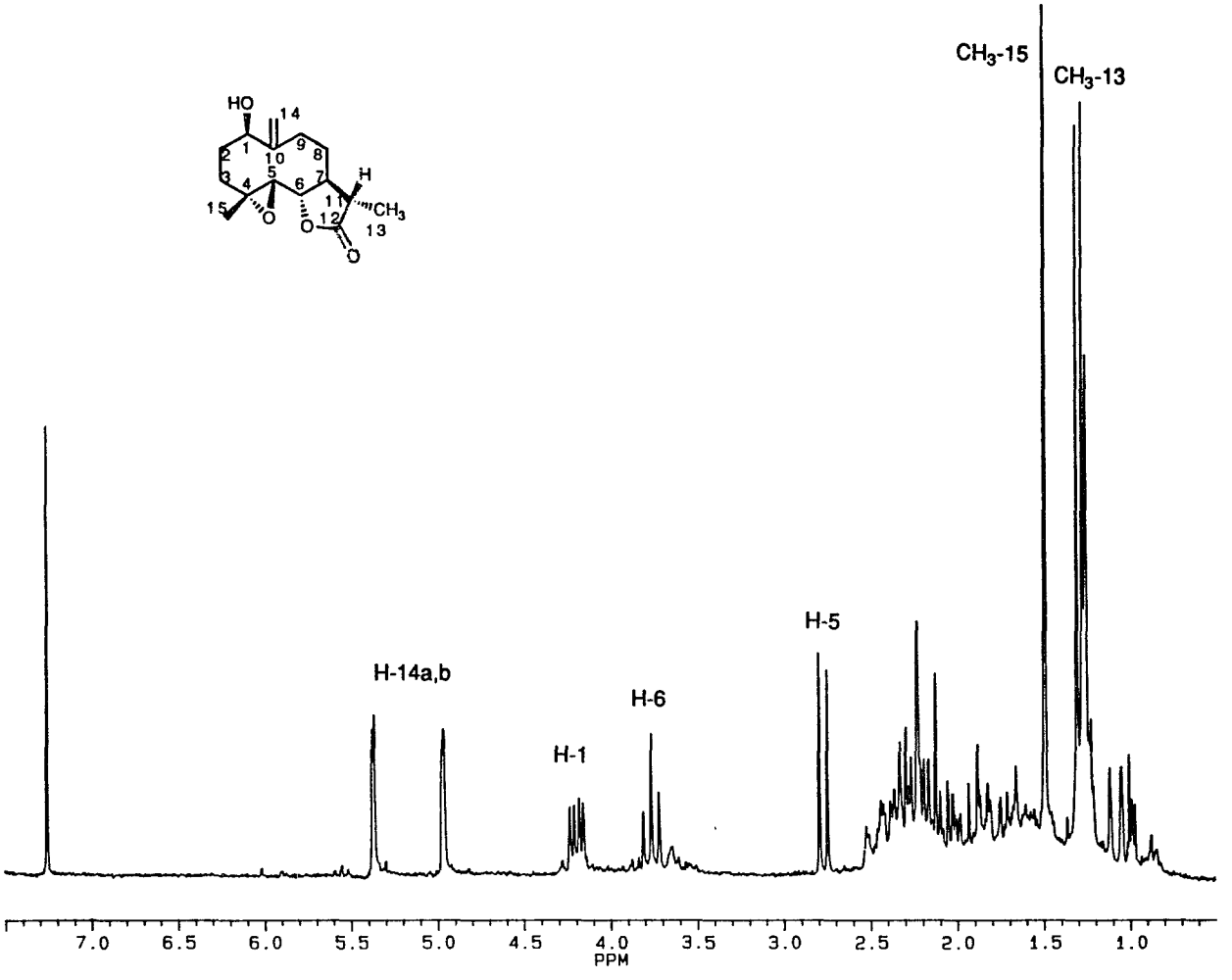


Figure 4.12. ^{1}H NMR spectrum of compound 26 in $CDCl_3$.

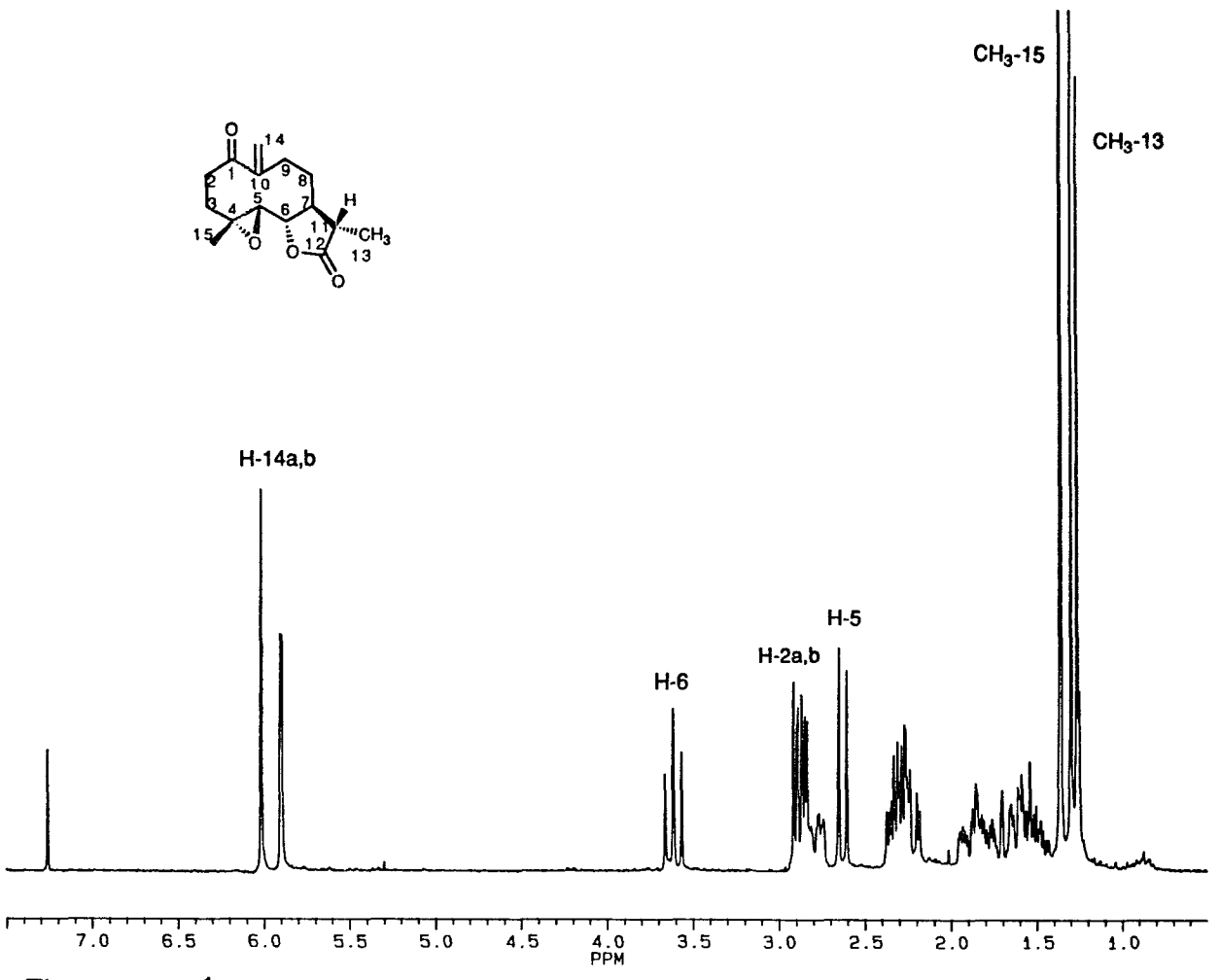


Figure 4.13. ^{1}H NMR spectrum of compound 27 in $CDCl_3$.

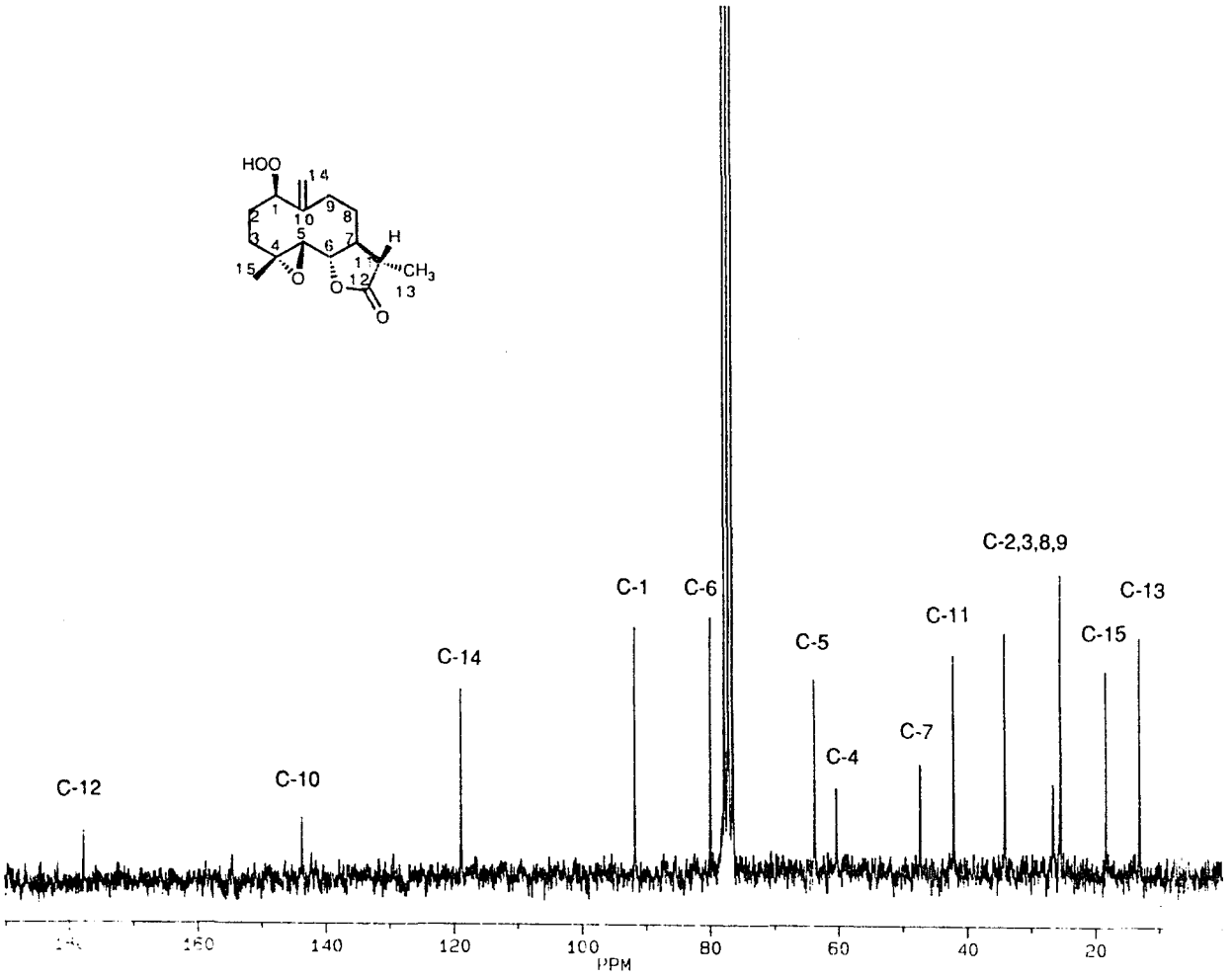


Figure 4.14. ^{13}C NMR spectrum of compound 25 in $CDCl_3$.

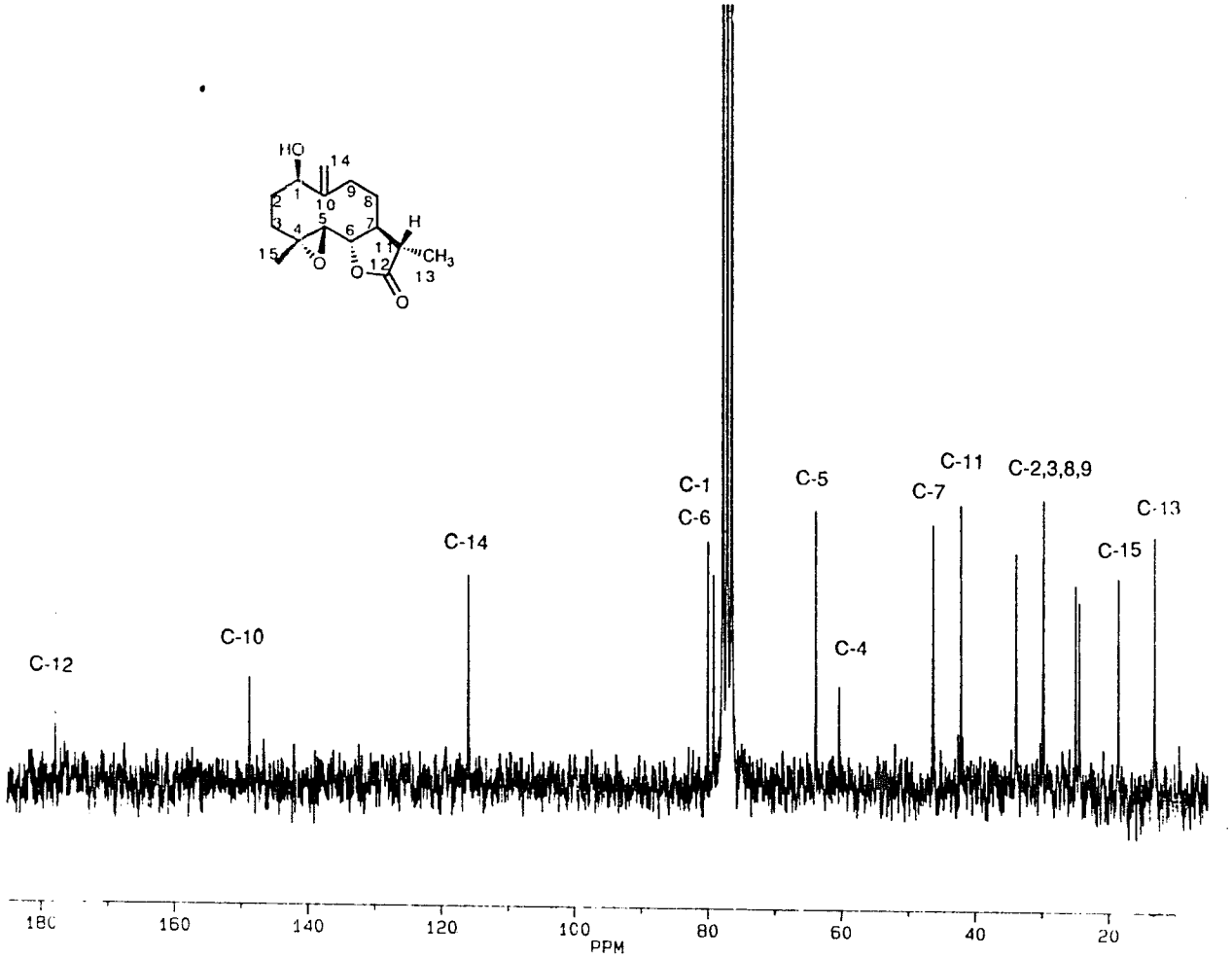


Figure 4.15. ^{13}C NMR spectrum of compound 26 in $CDCl_3$.

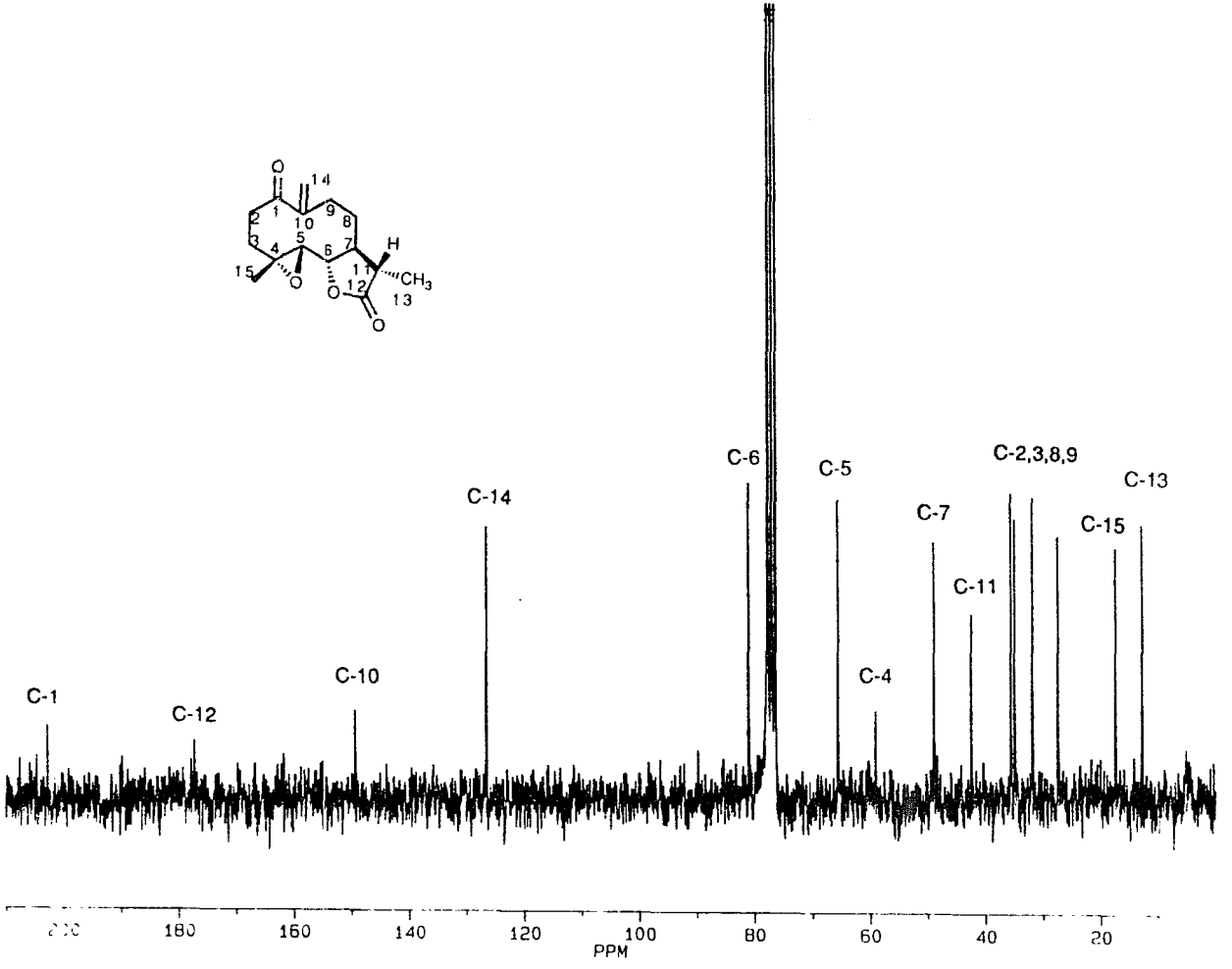


Figure 4.16. ^{13}C NMR spectrum of compound 27 in $CDCl_3$.

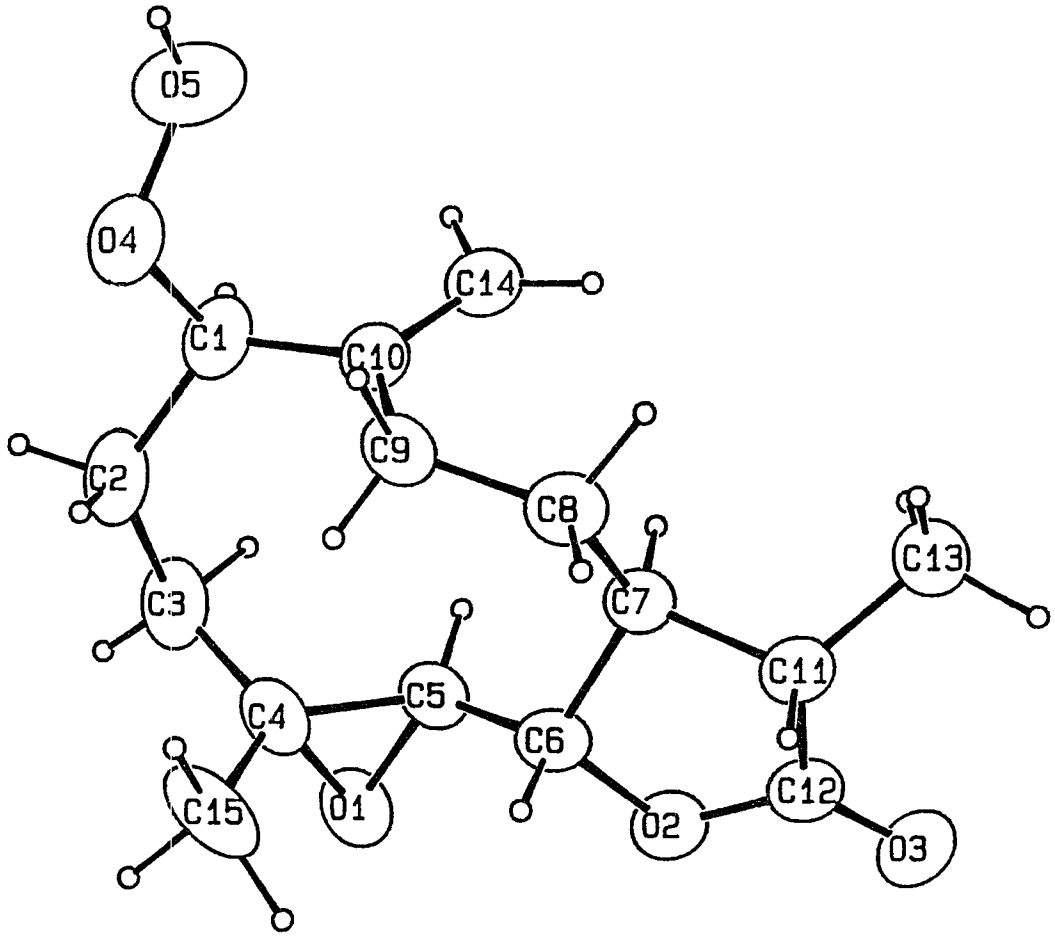


Figure 4.17. Molecular structure of compound **25**.

References

1. El-Feraly, F.S.; Chan, Y.-M.; Fairchild, E. H.; Doskotch, R. W. *Tetrahedron Lett.* **1977**, 1973-6.
2. Doskotch, R.W.; El-Feraly, F.S.; Fairchild, E.H.; Huang, C. *J. Org. Chem.* **1977**, *42(22)*, 3614-8.
3. Doskotch, R.W.; El-Feraly, F.S.; Fairchild, E.H.; Huang, C.-T. *J. Chem. Soc. Chem. Commun.* **1976**, 402-3.
4. El-Feraly, F.S.; Chan, Y.-M.; Capiton, G.A.; Doskotch, R.W.; Fairchild, E. H. *J. Org. Chem.* **1979**, *44(22)*, 3952-5.
5. Denny, R.W.; Nickon, A. Org. React. **1973**, *20*, 133-336.
6. Senda, Y.; Ishiyama, J.-i.; Imaizumi, S. *Tetrahedron Lett.* **1978**, 1805-8.
7. Sathe, R.N.; Kulkarni, G.H.; Kelkar, G.R.; Das, K.G. *Organic Mass Spectroscopy* **1969**, *2*, 935-45.
8. Isolated from the dichloromethane extract of the aerial parts of *Ambrosia artemisiifolia.*

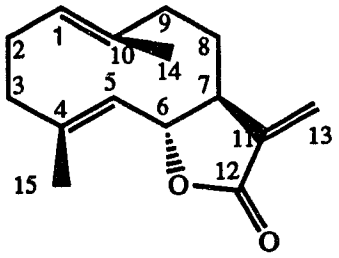
Part D. Attempted Biomimetic Conversion of a Germacrolide to a Heliangolide via Selenium Dioxide Oxidation

Introduction

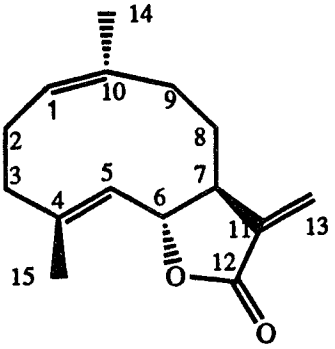
The largest group of naturally occurring sesquiterpene lactones are the germacranolides which contain a cyclodecadiene skeleton. The germacranolides are divided into four sub-groups based on the configuration of the double bonds in the cyclodecadiene ring (Scheme 4.8).[1,2] The germacrolides contain a cyclodecadiene ring system in which both double bonds are trans. The melampolides have a 1(10)-cis double bond and a 4(5)-trans-double bond. The heliangolides have a 1(10)-trans double bond and a 4(5)-cis double bond. The cis,cis-germacranolides contain two cis double bonds in the cyclodecadiene ring.

The biosynthetic pathway for the formation of sesquiterpene lactones is not known, however, it has been proposed that the germacranolides are biogenetically derived from (E,E)-farnesyl pyrophosphate (Scheme 4.9).[3] Cyclization of (E,E)-farnesyl pyrophosphate would generate the naturally occurring triene **(29)**. Allylic oxidation of triene **(29)** would generate alcohol **(31)**. Further oxidation of alcohol **(31)** to the carboxylic acid **(32)** followed by C-6 or C-8 oxidation and lactonization would generate the 12,6- and 12,8-lactonized germacranolides. The detailed steps and the sequence of these biogenetic transformations are not known.

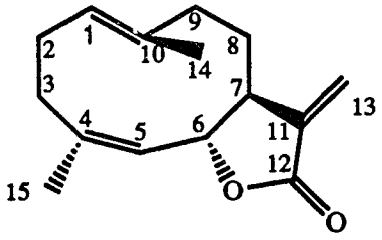
The melampolides, heliangolides, and the cis,cis-germacranolides may be derived from their respective (Z,E)-, (E,Z)-, and (Z,Z)-farnesyl pyrophosphates. They may equally well be derived from enzymatic or photochemical isomerizations of the double bonds of the trans,trans-cyclodecadiene **(29)** or any of its proposed derivatives.[3] Since many of the naturally occurring melampolides, heliangolides, and cis,cis-germacranolides contain oxygenated functionalities at C-14 or C-15, the double bond isomerizations (from **29** or its derivatives) may involve oxidative reactions.



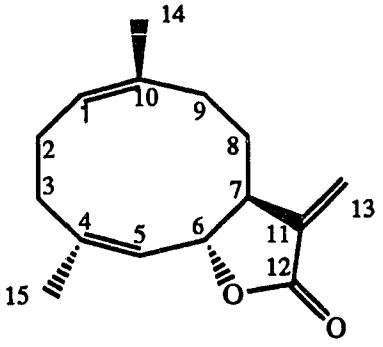
germacrolide



melampolide

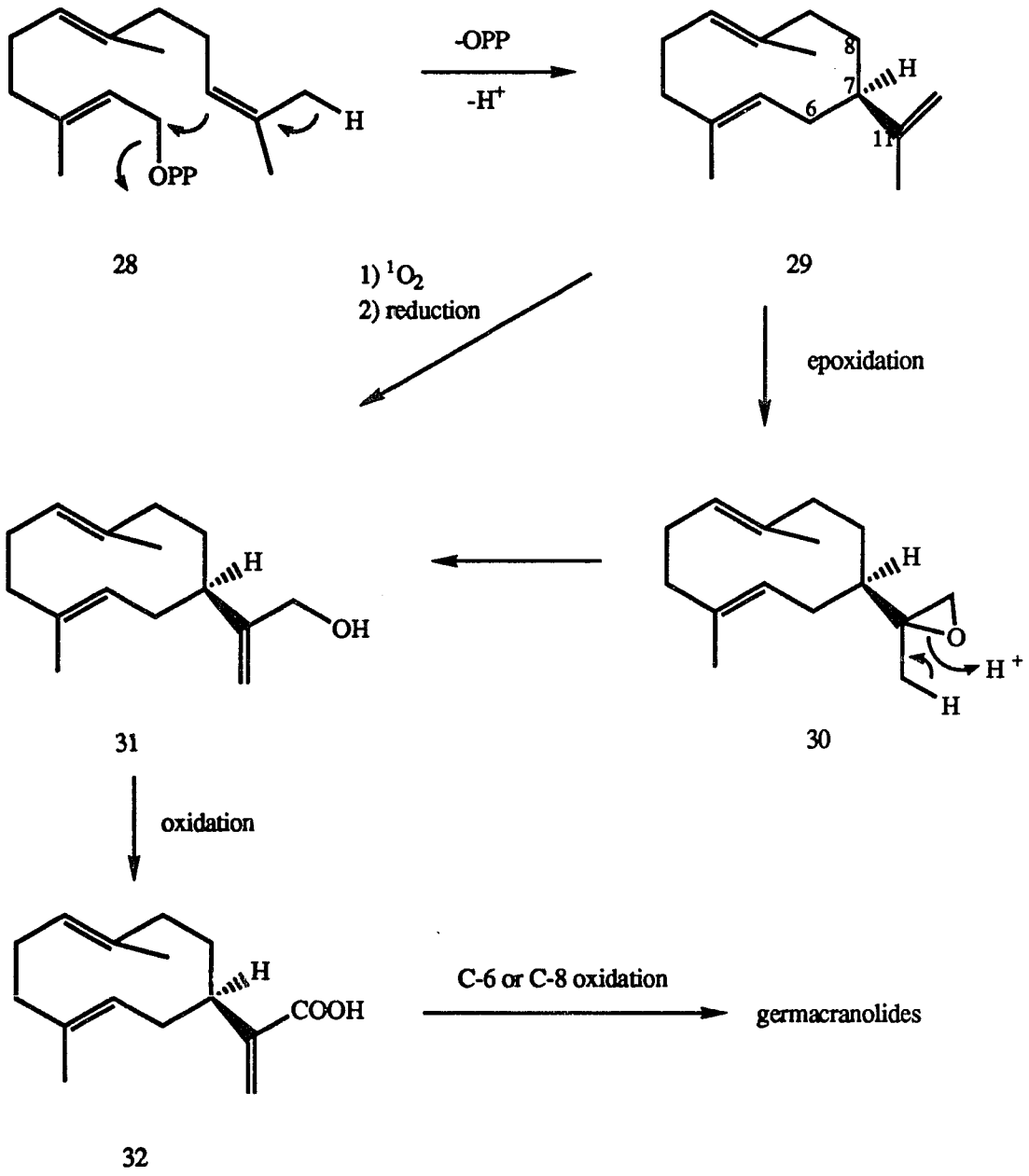


heliangolide



cis,cis-germacranolide

Scheme 4.8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-H+
H
OPP
8
7
H
6
11
28
29
1) 1O2
2) reduction
epoxidation
H
OH
H
O
H +
H
31
30
oxidation
H
COOH
C-6 or C-8 oxidation
germacranolides
32

Scheme 4.9

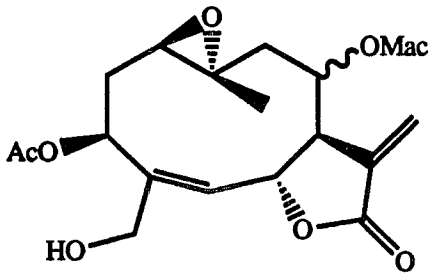
To test the latter hypothesis, oxidative chemical transformations have been attempted to convert germacrolides into the other three sub-groups of germacranolides. Reactions with selenium dioxide (SeO_2) and SeO_2/tert-butyl hydroperoxide (tBuOOH) provide in good yields melampolides as well as cis,cis-germacranolides.[4-7] These *in vitro* transformations provide indirect evidence for the possible biosynthetic routes for C-14 oxygenated melampolides and the cis,cis-germacranolides. The oxidations with SeO_2 are highly regio- and stereospecific reactions. Conformational changes occur as well as double bond isomerizatrions in these reactions which lead to conformations typically found in natural melampolides.

Natural heliangolides (like eriophyllin, Scheme 4.10) often have oxygenated functionalities at C-15, however, an *in vitro* transformation of a germacrolide to a heliangolide has not been carried out to date.

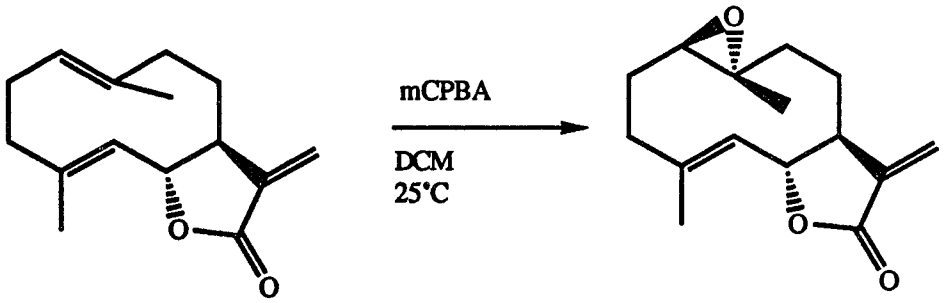
Results and Discussion

We reasoned that protecting or blocking attack at the 1,10-double bond of a germacrolide might allow for specific oxidation to occur at C-15 generating the first biomimetic transformation of a germacrolide to a heliangolide (Scheme 4.11). The 1,10-double bond was protected by epoxidation (with meta-chloroperoxybenzoic acid) under standard conditions to prevent transannular cyclizations.[8] An attempt was made to oxidize 1,10-epoxycostunolide (**34**) using catalytic amounts of SeO_2 in the presence of tBuOOH.[9]

The reaction product mixture was separated by silica gel vacuum liquid chromatography (VLC)[10] eluting with mixtures of dichloromethane (DCM) and acetone. Santamarine (**36**) and reynosin (**37**), two naturally occurring



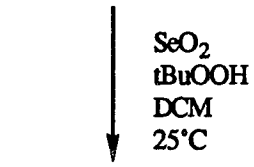
Scheme 4.10

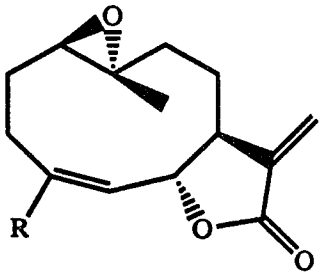


33

34

germacrolide





R = CHO
R = CH_2OH

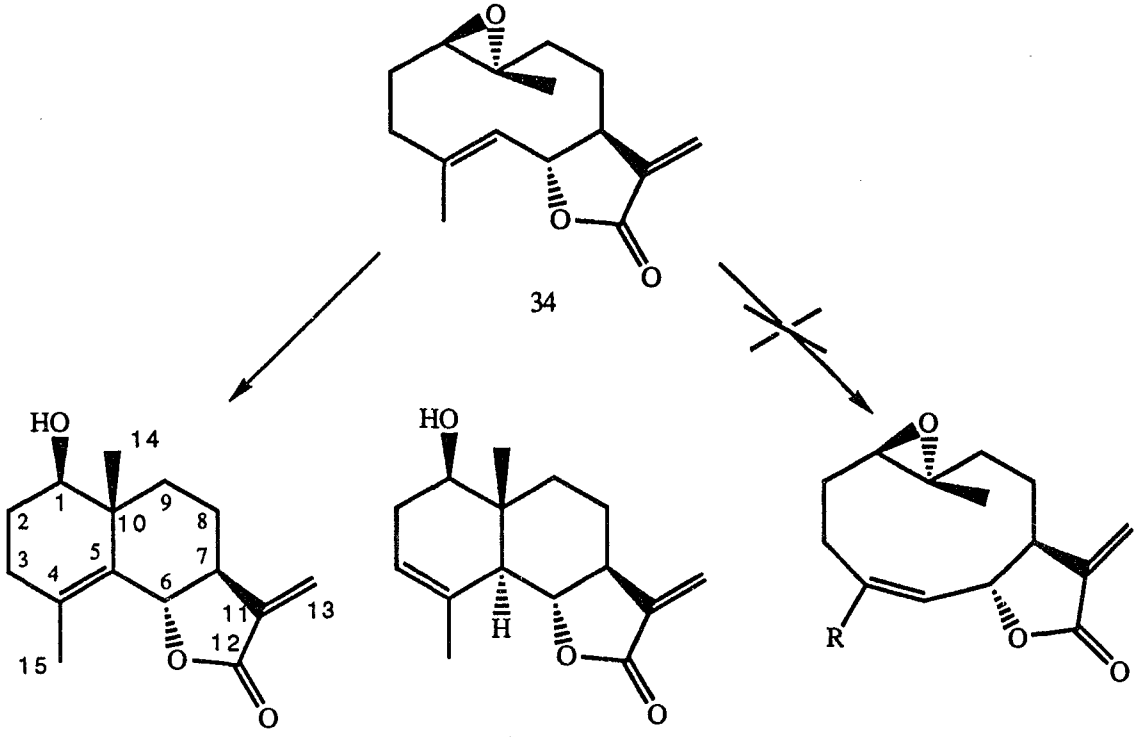
heliangolide

Scheme 4.11

sesquiterpene lactones, were isolated as the major products from the reaction mixture (Scheme 4.12). The 4,5-double bond isomer of santamarine (**36**) was isolated as a minor product, and some unreacted 1,10-epoxycostunolide (**34**) was recovered. The ^{1}H NMR data for compounds **36** and **37** is identical to that already reported in the literature for these compounds.[11,12] Compound **35** was identified by its ^{1}H NMR spectrum which shows two methyl singlets, one at 1.88ppm (C-15) and one at 1.12ppm (C-14), indicating a eudesmanolide skeleton. No olefinic proton signals were present in the NMR spectrum of compound **35**. Also, the signal for H-6 is a doublet (indicating coupling to only 1 proton, H-7) at 4.54ppm which is shifted about 0.5ppm downfield from H-6 of compound **36** indicating the allylic nature of H-6 in compound **35**. No allylic oxidation products were isolated. Apparently, acidic conditions are generated in the SeO_2/tBuOOH reaction and transannular cyclization occurs.

It was reasoned that the addition of a base to the reaction mixture (to act as a proton sponge) would inhibit acid-catalyzed cyclizations, thus allowing for the desired allylic oxidations to occur. 1,10-Epoxycostunolide (**34**) was reacted under the same conditions (tBuOOH plus a catalytic amount of SeO_2) in the prescence of pyridine. No reaction occurred (no allylic oxidation and no cyclization); only compound **34** was isolated even after four days of stirring at room temperature. Since no cyclization of compound **34** occurred in the prescence of pyridine, use of such bases might be a convenient way to stabilize these acid-sensitive compounds.

The allylic oxidation of compound **34** was attempted using stoichiometric amounts of SeO_2 (instead of catalytic) in ethanol at reflux.[13] Oxidation evidently occurred during the reaction as evidenced by the formation of red, shiny, metallic selenium on the sides of the reaction flask. After silica gel column chromatography, three products were isolated from the reaction mixture: the 4,5-double bond isomer of santamarine (**35**), 3-hydroxyreynosin (**38**), and 2,5-dihydroxysantamarine

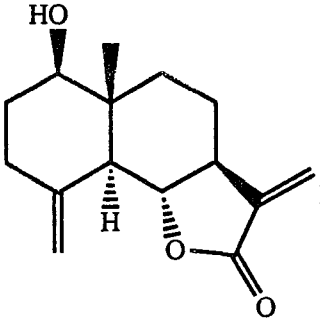


34

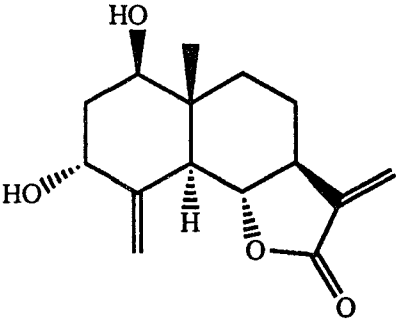
35

36

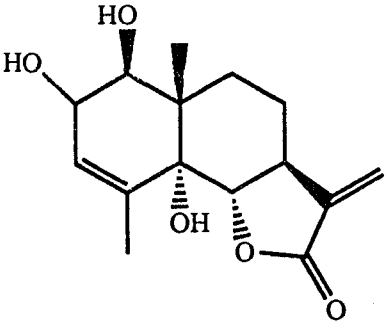
R = CHO
R = CH_2OH



37



38



39

Reaction Conditions	Products
1. SeO_2/tBuOOH/DCM/25°C/24hrs	35,36
2. SeO_2/tBuOOH/pyridine/DCM/25°C/4days	-----
3. SeO_2/95% ethanol/reflux/5hrs	35,38,39
4. SeO_2/95% ethanol/pyridine/reflux/4hrs	38,39

Scheme 4.12

(**39**) (Scheme 4.12). Under these conditions cyclization occurs first followed by allylic oxidation. The ^{1}H NMR spectra of compound **38** was very similar to that of reynosin (**37**) except for a one proton doublet of a doublet at 4.38ppm indicating an allylic proton geminal to an OH group (at C-3). The narrow couplings (~3Hz) suggest that H-3 bisects the two C-2 protons which requires that H-3 is beta. The ^{1}H NMR spectrum of compound **39** was similar to santamarine (**36**) except for a one proton broad multiplet at 4.22ppm indicating an allylic proton geminal to an OH group (at C-2). The multiplicity of the signal for H-6 at 4.08ppm is a doublet indicating only one proton on an adjacent carbon. The multiplicity of H-6 and the relative polarity of the molecule indicated the prescence of an OH group at C-5. Compounds **38** and **39** apparently are formed from allylic oxidation of cyclization products of compound **34**.

The allylic oxidation of compound **34** using stoichiometric amounts of SeO_2 in ethanol at reflux was also attempted in the prescence of pyridine to see if cyclization could be prevented, but allow allylic oxidation to occur. From this reaction, products **38** and **39** were isolated once more (Scheme 4.12).

The 1,10-epoxy-derivatives appear to be too labile in order to allow allylic oxidation to occur. If another protecting group can be found for the 1,10-double bond which is not as labile, then these transformations might be successful. In lieu of such a protecting group, regioselective oxidation conditions must be found. Since it is known that an allylic OH group can often direct attack at double bonds (like in the Sharpless asymmetric method of epoxidation[14]) we reasoned that a C-6-OH germacrolide, like 6-epi-desacetyl-laurenobiolide (**40**), might regioselectively be oxidized at C-15 with tBuOOH/SeO_2.

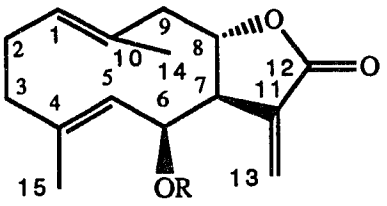
From an attempted allylic oxidation of compound **40** with tBuOOH and catalytic SeO_2 four products were isolated: the 4,5- and 1,10-mono-epoxides (**42**),

(**43**) and two melampolides (**44**), and (**45**) (Scheme 4.13). For the melampolides, allylic oxidation took place at both C-14 and C-15. The aldehyde function at C-15 reacted with the OH group at C-6 to form a hemi-acetal in both cases. In order to try to isolate any mono-allylic oxidation products the reaction time was shortened from 3hrs to 30 minutes. However, after 30 minutes only the 4,5-epoxide (**42**) was isolated.

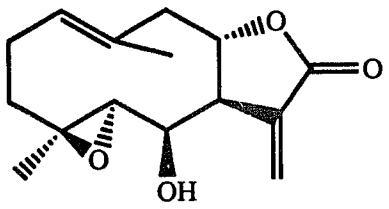
It is unusual for epoxidations to occur under these conditions.[9] It is unclear whether the epoxidations are carried out by SeO_2 or the secondary oxidant present tBuOOH. The flexibility of the medium ring may play a role in the preference for epoxidation over allylic oxidation.

The ^1H NMR data of compound **42** is identical to that reported by Quijano et al.[15] The one proton doublet of a doublet at 2.53ppm for compound **43** indicates a 1,10-epoxide. The ^1H NMR data of compound **44** exhibits an aldehyde signal at 9.51ppm and a hemiacetal singlet signal at 5.73ppm. The ^1H NMR data of compouund **45** also shows a hemiacetal singlet signal at 6.05ppm as well as a two proton doublet of a doublet at 4.14ppm (CH_2OH).

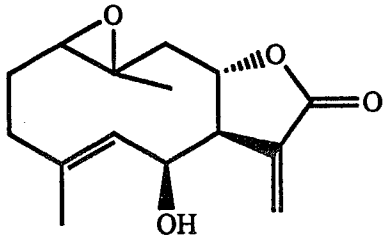
Chhabra and Hayano[16] showed that in a solid matrix (SeO_2 impregnated in silica gel) with tBuOOH only allylic methyl groups are oxidized and no undesired epoxidations occur. Since this is the selectivity that is desired, this methodology was used to oxidize 6-epi-laurenobiolode (**41**). From this product mixture two compounds were isolated; the melampolide (**46**) and the heliangolide (**47**) (Scheme 4.13). The ^1H NMR spectra of both products show the disappearance of a methyl singlet and the appearance of a two proton signal at 4.20 or 4.06ppm indicating allylic methyl group oxidation to CH_2OH. Compound **46** was identified as a melampolide based on the sharp signals for H-13a,b (doublets) with coupling constants of 3Hz. Small allylic coupling constants $J_{7,13}$ of 1 or 2 Hz suggests either



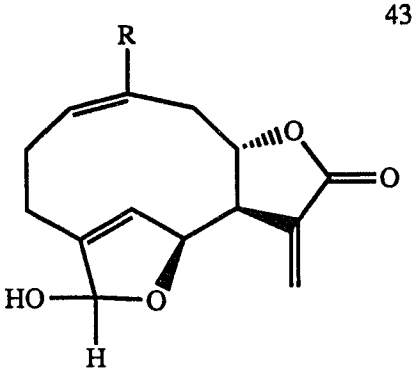
40 R = H
41 R = Ac



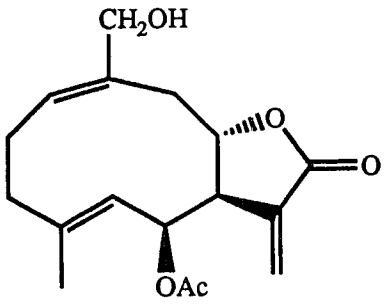
42



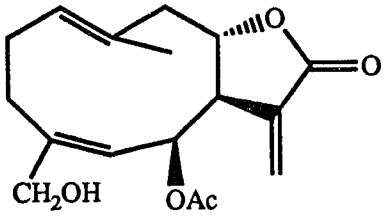
43



44 R = CHO
45 R = CH_2OH



46



47

Scheme 4.13

a cis-fused lactone or a trans-fused heliangolide type compound.[17-19] Compound **47** was identified as a heliangolide based on the very broad NMR signals for H-13a,b (coupling constants are not distinguishable).[1]

Experimental Section

^1H NMR spectra were recorded on either a Bruker-AC200 or an AM-400 spectrometer in $CDCl_3$ using $SiMe_4$ as an internal standard. Column chromatographic separations were made on silica gel (60-200M, J.T. Baker Chemical Co.) Vacuum liquid chromatographic (VLC) separations were made on silica gel (MN Kieselgel G). Costunolide (**33**) was isolated by VLC from Costus Resinoid (Pierre Chauvet, S.A.) 6-Epi-desacetyl-laurenobiolide (**40**), isolated from *Montanoa grandiflora*,[15] was graciously provided by Dr. Leo Quijano.

Epoxidation of costunolide (33). Costunolide (**33**) (100mg, 0.43mmol) was dissolved in 10ml of dichloromethane (DCM). Sodium acetate (100mg) was added to the solution to buffer the reaction and prevent cyclization. Meta-chloroperoxybenzoic acid (mCPBA, 97mg, 0.43mmol) was also added to the solution, which was stirred at room temp. The reaction was monitored by TLC and after 15min. all of the costunolide had reacted. The DCM solution was filtered by gravity to remove NaOAc, and then was washed with 5% aq. Na_2CO_3 (25ml x 2) and distilled water (40ml x 2). The organic phase was dried over anhydrous Na_2SO_4, filtered, and the solvent was evaporated yielding 105mg (98%) of a white solid whose ^1H NMR spectrum is identical to that reported for 1,10-epoxycostunolide (**34**).[8]

1,10-epoxycostunolide (34) ^1H NMR (Fig. 4.18): δ 6.26 (d,1H,C_{13}-Hb,J=3Hz), 5.50 (d,1H,C_{13}-Ha,J=3Hz), 5.28 (d,1H,C_5-H,J=10Hz), 4.61 (dd,1H,C_6-H,J=10Hz), 2.70 (dd,1H,C_1-H,J=2,11Hz), 1.63 (d,3H,C_{15}-

CH_3,J=2Hz), 1.13 (s,3H,C_{14}-CH_3). Compound **34** is not a stable compound and readily undergoes cyclization (as evidenced by its ^1H NMR spectrum, Figure 4.18). To minimize this reaction, the epoxidation of compound **33** was repeated, however, compound **34** was not isolated from the DCM solution as before, but was used in solution for the attempted allylic oxidations.

Attempted allylic oxidation of 1,10-epoxycostunolide (34) with tBuOOH and catalytic SeO_2. The DCM solution of compound **34** was added dropwise by pipet to a suspension of SeO_2 (23.9mg, 0.215mmol) and 70% tBuOOH (0.11ml, 0.43mmol) in 1ml of DCM. The solution was stirred at room temp. for 24hrs, after which time the solution was dried over anhydrous Na_2SO_4, filtered, and the solvent was evaporated. The products were separated by VLC eluting with mixtures of DCM and acetone. The 400MHz ^1H NMR spectra of the eluted fractions show the prescence of two major products, santamarine (**36**) and reynosin (**37**), a minor product compound **35**, and some unreacted starting compound **34**. The ^1H NMR data for the known sesquiterpene lactones **36** and **37** are identical to that reported in the literature.[11]

Compound (35) ^1H NMR (Fig. 4.19): δ 6.15 (d,1H,C_{13}-Hb,J=3Hz), 5.48 (d,1H,C_{13}-Ha,J=3Hz), 4.54 (d,1H,C_6-H,J=11Hz), 3.53 (m,1H,C_1-H), 1.88 (s,3H,C_{15}-CH_3), 1.12 (s,3H,C_{14}-CH_3).

Attempted allylic oxidation of 1,10-epoxycostunolide (34) with tBuOOH and catalytic SeO_2 in the prescence of pyridine. The oxidation was carried out as before except 1ml of pyridine was added to the suspension before addition of the DCM solution of compound **34**. The reaction was monitored by TLC for four days. The prescence of pyridine makes TLC analysis difficult because pyridine appears as a deep purple spot under UV light. After work-up as before, ^1H NMR analysis of the product showed the prescence of only unreacted

1,10-epoxycostunolide (**34**).

Attempted allylic oxidation of 1,10-epoxycostunolide (34) with stoichiometric SeO_2. SeO_2 (99mg) was added to a solution of compound **34** dissolved in 10ml of 95% ethanol. The solution was refluxed for 5hrs. Red metallic selenium began to appear on the sides of the round-bottom flask. The solution was filtered and the solvent was evaporated, generating an orange, oily residue. The residue was dissolved in water and extracted with DCM (10ml x 4). The DCM solution was washed with 5% aq. $NaHCO_3$ (2 x 10ml), and water (2 x 10ml), dried over anhydrous Na_2SO_4, filtered, and the solvent evaporated. Silica gel column chromatography (eluting with mixtures of DCM and acetone) was used to separate compounds **35**, **38**, and **39**.

3-hydroxyreynosin (38) ^1H NMR (Fig. 4.20): δ 6.10 (d,1H,C_{13}-Hb,J=3Hz), 5.43 (d,1H,C_{13}-Ha,J=3Hz), 5.19 (s,1H,C_{14}-H), 5.03 (s,1H,C_{14}-H), 4.38 (dd,1H,C_3-H,J=3Hz), 4.02 (dd,1H,C_6-H,J=11Hz), 3.94 (dd,1H,C_1-H,J=5,12Hz), 2.77 (d,1H,C_5-H,J=11Hz), 0.79 (s,3H,C_{15}-CH_3).

2,5-dihydroxysantamarine (39) ^1H NMR (Fig. 4.21): δ 6.08 (d,1H,C_{13}-Hb,J=3Hz), 5.49 (m,br,1H,C_3-H), 5.40 (d,1H,C_{13}-Ha,J=3Hz), 4.22 (m,br,1H,C_2-H), 4.08 (d,1H,C_6-H,J=11Hz), 3.33 (m,1H,C_1-H), 1.90 (s,3H,C_{14}-CH_3), 0.93 (s,3H,C_{15}-CH_3).

Attempted allylic oxidation of 1,10-epoxycostunolide (34) with stoichiometric SeO_2 in the prescence of pyridine. The reaction was carried out as above, except 1ml of pyridine was added to the solution which was refluxed for 4hrs. Compounds **38** and **39** were isolated from the product mixture.

6-epi-desacetyl-laurenobiolide (40)[15] ^1H NMR (Fig. 4.22): δ 6.42 (d,1H,C_{13}-Hb,J=3Hz), 5.71 (d,1H,C_{13}-Ha,J=2Hz), 4.85-5.15 (m,br,2H,C_1-H,C5-H), 4.50-4.81 (m,br,2H,C_6-H,C_8-H), 1.63 (s,3H,C_{15}-CH_3), 1.54

(s,3H,C_{14}-CH_3).

Attempted allylic oxidation of 6-epi-descaetyl-laurenobiolide (40) with tBuOOH and catlytic SeO_2. A DCM solution of compound **40** was added dropwise by pipet to a suspension of SeO_2 (28mg, 0.25mmol), and 70% tBuOOH (0.32ml, 1mmol) in 1ml of DCM. This solution was stirred at room temp. for 3hrs., after which the solution was dried over anhydrous Na_2SO_4, filtered, and the solvent evaporated. The products were separated by dry column (silica gel) chromatography.[20] Both the 4,5- and the 1,10-epoxides **(42)**, **(43)** were separated from the product mixture. The ^1H NMR data for compound **42** is identical to that reported by Quijano et al.[15] Two hemiacetals were also isolated: one with an aldehyde functionality at C-14 **(44)** and one with an alcohol functionality at C-14 **(45)**.

Epoxide (42)[15] ^1H NMR (Fig. 4.24): δ 6.48 (d,1H,C_{13}-Hb,J=3Hz), 5.65 (d,1H,C_{13}-Ha,J=3Hz), 5.41 (dd,1H,C_1-H,J=8Hz), 4.77 (ddd,1H,C_8-H,J=12,4,4Hz), 3.77 (d,1H,C_6-H,J=8Hz), 2.90 (m,1H,C_7-H), 2.51 (d,1H,C_5-H,J=8Hz), 1.73 (s,3H,C_{14}-CH_3), 1.31 (s,3H,C_{15}-CH_3).

Epoxide (43) ^1H NMR (Fig. 4.25): δ 6.49 (d,1H,C_{13}-Hb,J=3Hz), 5.74 (d,1H,C_{13}-Ha,J=3Hz), 5.30 (d,1H,C_5-H,J=8Hz), 4.83 (m,1H,C_8-H), 4.76 (dd,1H,C_6-H,J=5,2Hz), 2.72 (m,1H,C_7-H), 2.53 (dd,1H,C_1-H,J=9,4Hz), 1.79 (s,3H,C_{15}-CH_3), 1.43 (s,3H,C_{14}-CH_3).

Aldehyde (44) ^1H NMR (Fig. 4.26): δ 9.51 (s,1H,C_{14}-CHO), 6.56 (dd,1H,C_1-H,J=9Hz), 6.37 (d,1H,C_{13}-Hb,J=3Hz), 5.95 (d,1H,C_5-H,J=4Hz), 5.73 (s,1H,C_{15}-H), 5.67 (d,1H,C_{13}-Ha,J=3Hz), 5.30 (d,1H,C_6-H,J=4Hz), 3.90 (dd,1H,C_8-H,J=7Hz).

Alcohol (45) ^1H NMR (Fig. 4.27): δ 6.35 (d,1H,C_{13}-Hb,J=3Hz), 6.05 (s,1H,C_{15}-H), 5.84 (d,1H,C_5-H,J=4Hz), 5.72 (d,1H,C_{13}-Ha,J=3Hz), 5.62

(d,1H,C_6-H,J=8Hz), 5.53 (dd,1H,C_1-H,J=3Hz), 4.14 (dd,2H,C_{14}-Ha,b), 3.84 (ddd,1H,C_8-H,J=6Hz), 2.90 (m,br,C_7-H).

The above reaction was repeated for a shorter period of time to see if only mono-allylic oxidation products could be isolated. Epoxide **(42)** was the only product isolated (some of compound **40** was recovered) after 30minutes.

Acetylation of 6-epi-desacetyl-laurenobiolide (40) Compound **40** (116mg) was acetylated in 10ml of acetic anhydride in the prescence of pyridine (5ml). The solution was stirred at room temp. for 24hrs., after which the solvent was evaporated. The acetate was analyzed by ^{1}H NMR and is identical to the data reported by Quijano et al.[15]

6-epi-laurenobiolide (41) ^{1}H NMR (Fig. 4.23): δ 6.33 (d,1H,C_{13}-Hb,J=3Hz), 5.76 (m,br,1H,C_6-H), 5.68 (d,1H,C_{13}-Ha,J=2Hz), (Quijano reports the previous signal at 5.29ppm) 4.50-5.08 (m,3H,C_1-H,C_5-H,C_8-H), 2.86 (m,1H,C_7-H), 1.93 (s,3H,OAc), 1.62 (s,3H,C_{14}- or C_{15}-CH_3), 1.60 (s,3H,C_{14}- or C_{15}-CH_3).

Preparation of SeO_2 supported on silica gel for selective allylic oxidations.[16] SeO_2 (50mg) was dissolved in 1ml of water. Methanol (50ml) was added to the solution. A slurry was prepared by adding 1gm of silica gel (60-200M, J. T. Baker Chemical Co.) to the solution. The solvent was evaporated generating an off-white powder.

Allylic oxidation of 6-epi-laurenobiolide (41) with tBuOOH and SeO_2 supported on silica gel. The SeO_2 impregnated silica gel (500mg) was mixed with 5ml of DCM and 0.19ml (3 equiv.) of 70% tBuOOH at room temp. A DCM solution of compound **41** was added dropwise to the suspension. After stirring at room temp. for 3hrs., the suspension was filtered by gravity. The DCM solution was washed with water and Na_2CO_3, dried over anhydrous Na_2SO_4, filtered, and the solvent evaporated. Dry column chromatography was used to

separate two alcohol products in very low yield from the product mixture, compounds **46** and **47**.

Alcohol (46) ^{1}H NMR (Fig. 4.28): δ 6.30 (d,1H,C_{13}-Hb,J=3Hz), 6.07 (dd,1H,C_1-H), 5.64 (d,1H,C_5-H), 5.57 (d,1H,C_{13}-Ha,J=3Hz), 4.90 (m,br,1H,C_1-H), 4.46 (m,br,1H,C_8-H), 4.20 (m,br,2H,C_{14}-Ha,b), 3.11 (m,1H,C_7-H), 2.01 (s,3H,OAc), 1.77 (s,3H,C_{15}-CH_3).

Alcohol (47) ^{1}H NMR (Fig. 4.29): δ 6.39 (s,br,1H,C_{13}-Hb), 5.72 (m,br,1H,C_6-H), 5.70 (s,br,1H,C_{13}-Ha), 5.19 (d,1H,C_5-H,J=8Hz), 5.03 (m,1H,C_1-H), 4.81 (m,1H,C_8-H), 4.06 (m,2H,C_{15}-Ha,b), 2.95 (m,1H,C_7-H), 1.98 (s,3H,OAc), 1.61 (s,3H,C_{14}-CH_3).

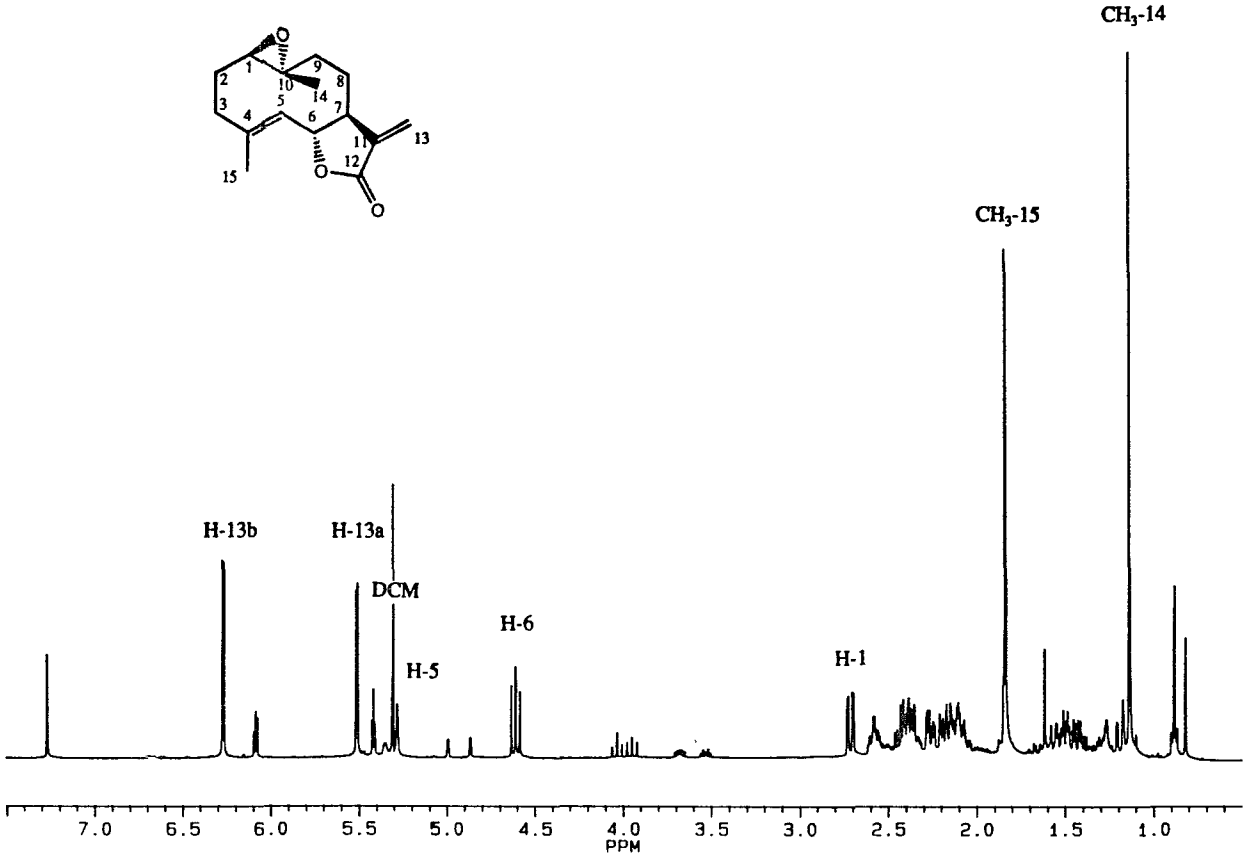


Figure 4.18. ^{1}H NMR spectrum of compound **34** in $CDCl_3$.

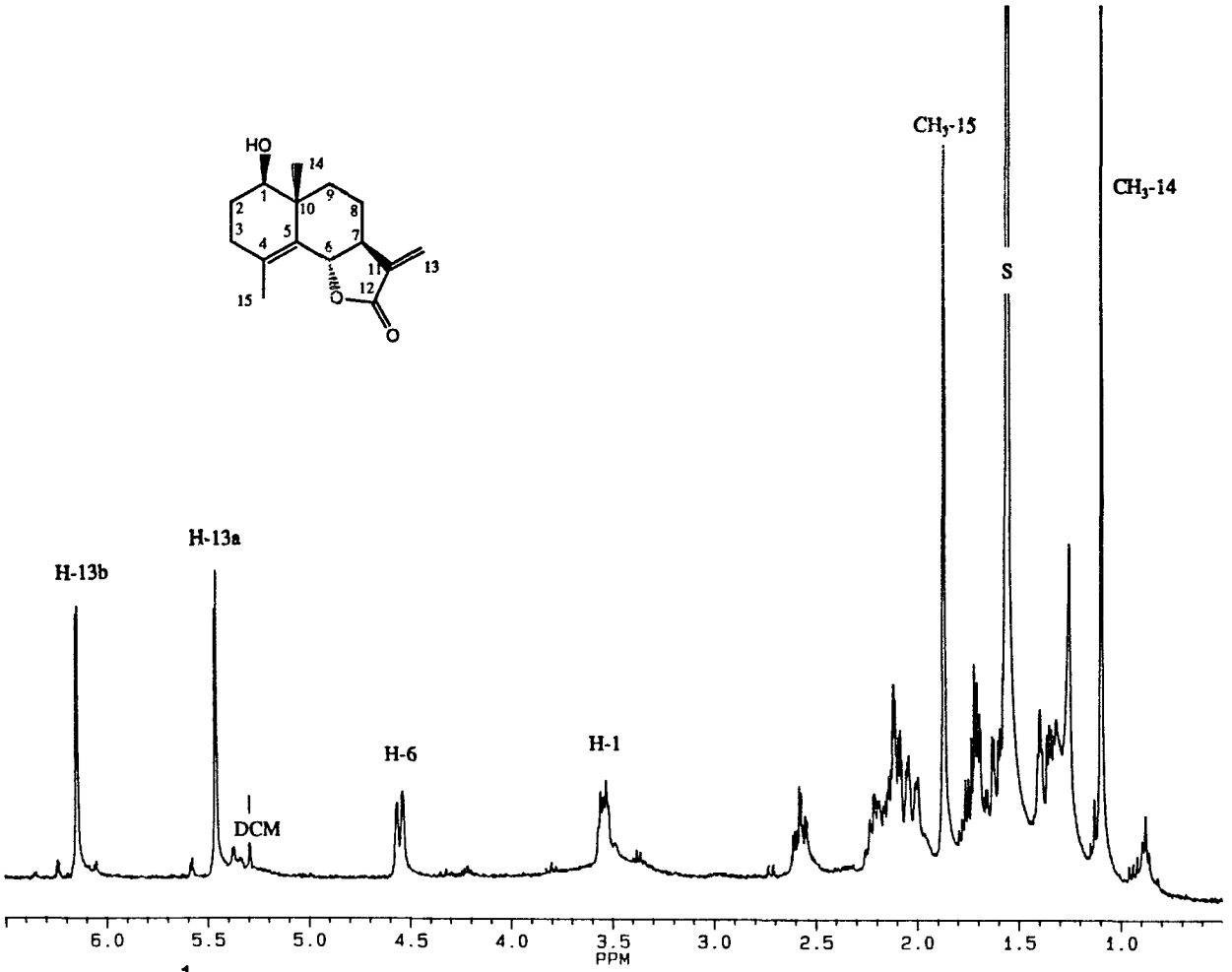


Figure 4.19. ^{1}H NMR spectrum of compound **35** in $CDCl_3$.

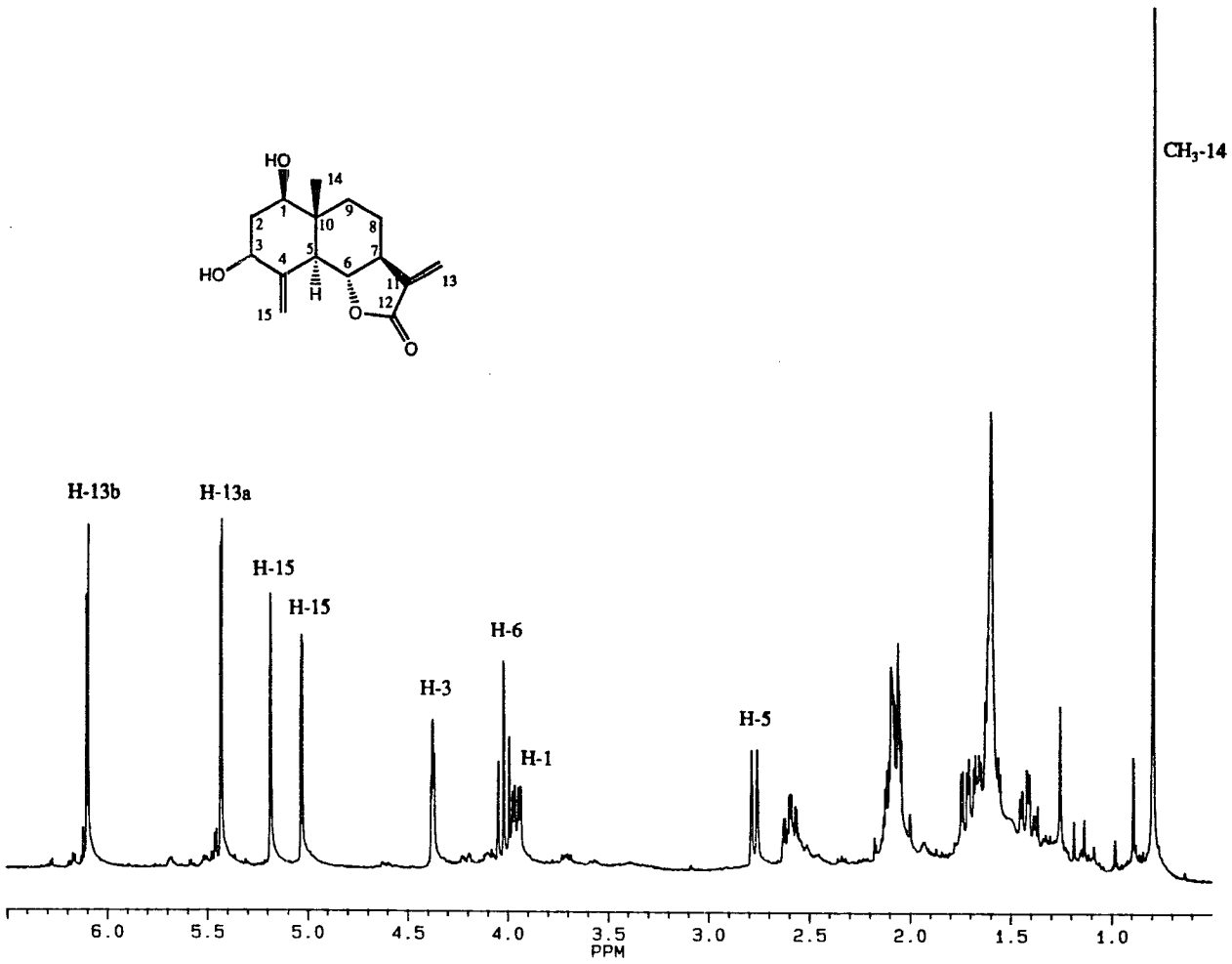


Figure 4.20. ^{1}H NMR spectrum of compound **38** in $CDCl_3$.

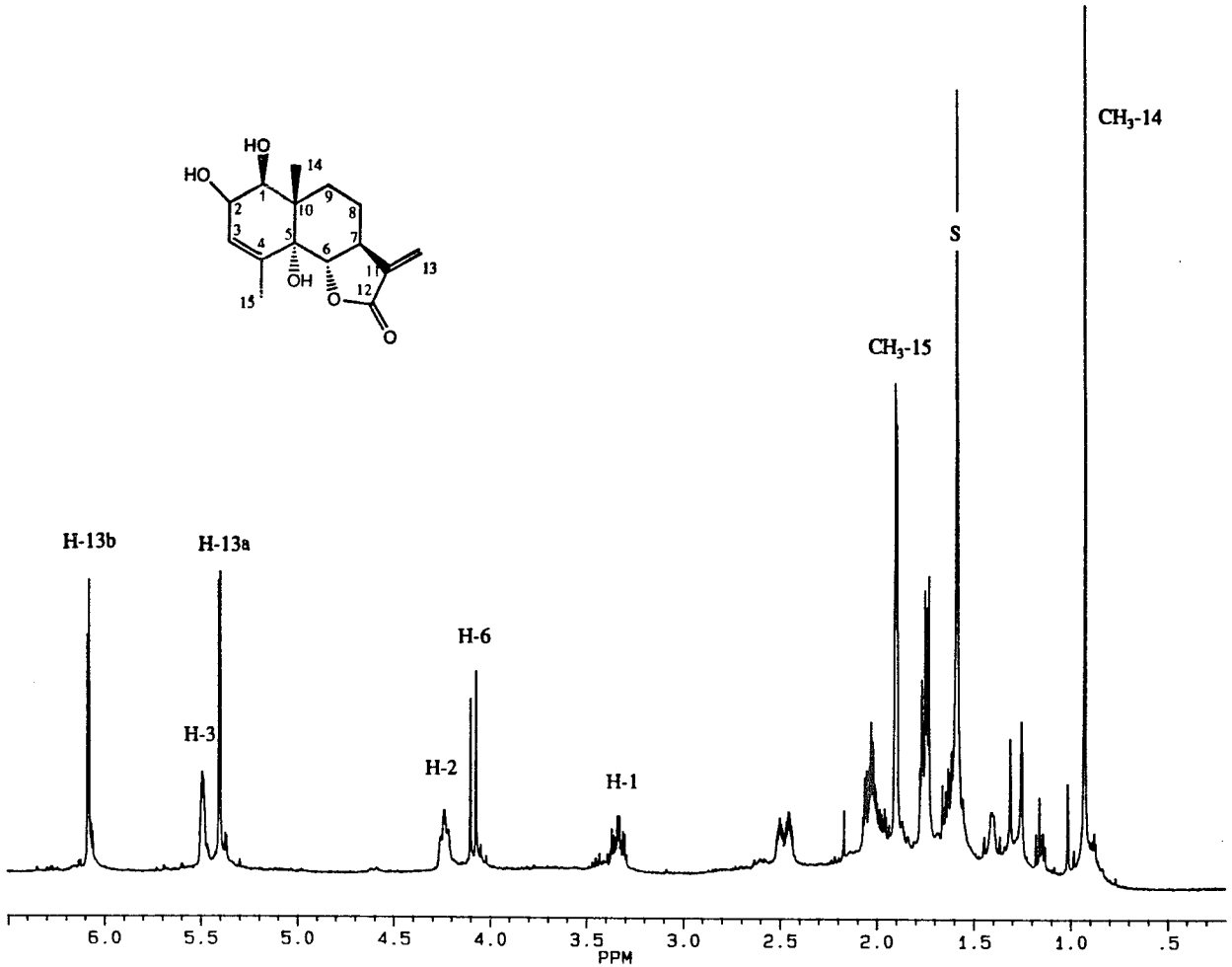


Figure 4.21. ^{1}H NMR spectrum of compound **39** in $CDCl_3$.

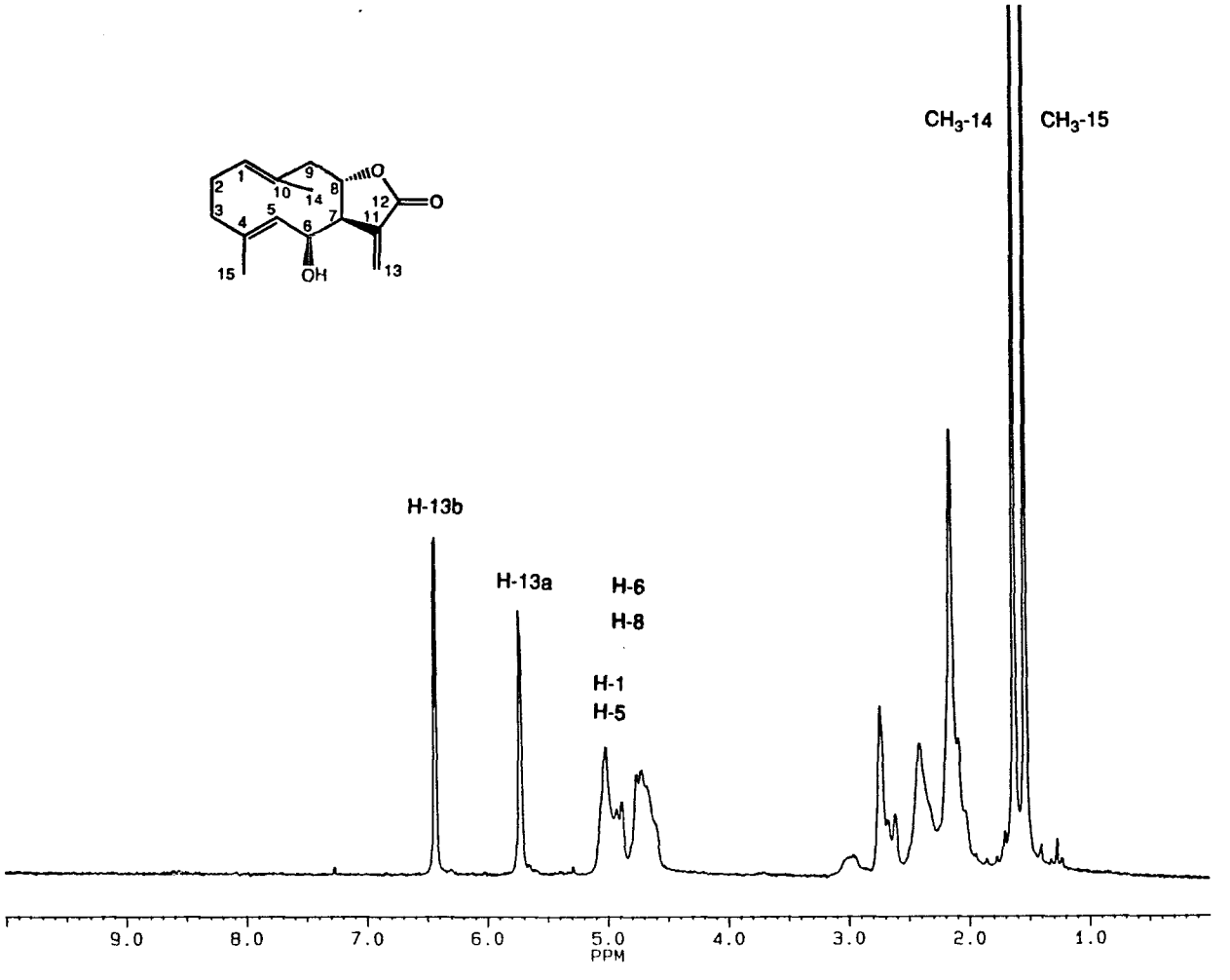


Figure 4.22. ^{1}H NMR spectrum of compound **40** in $CDCl_3$.

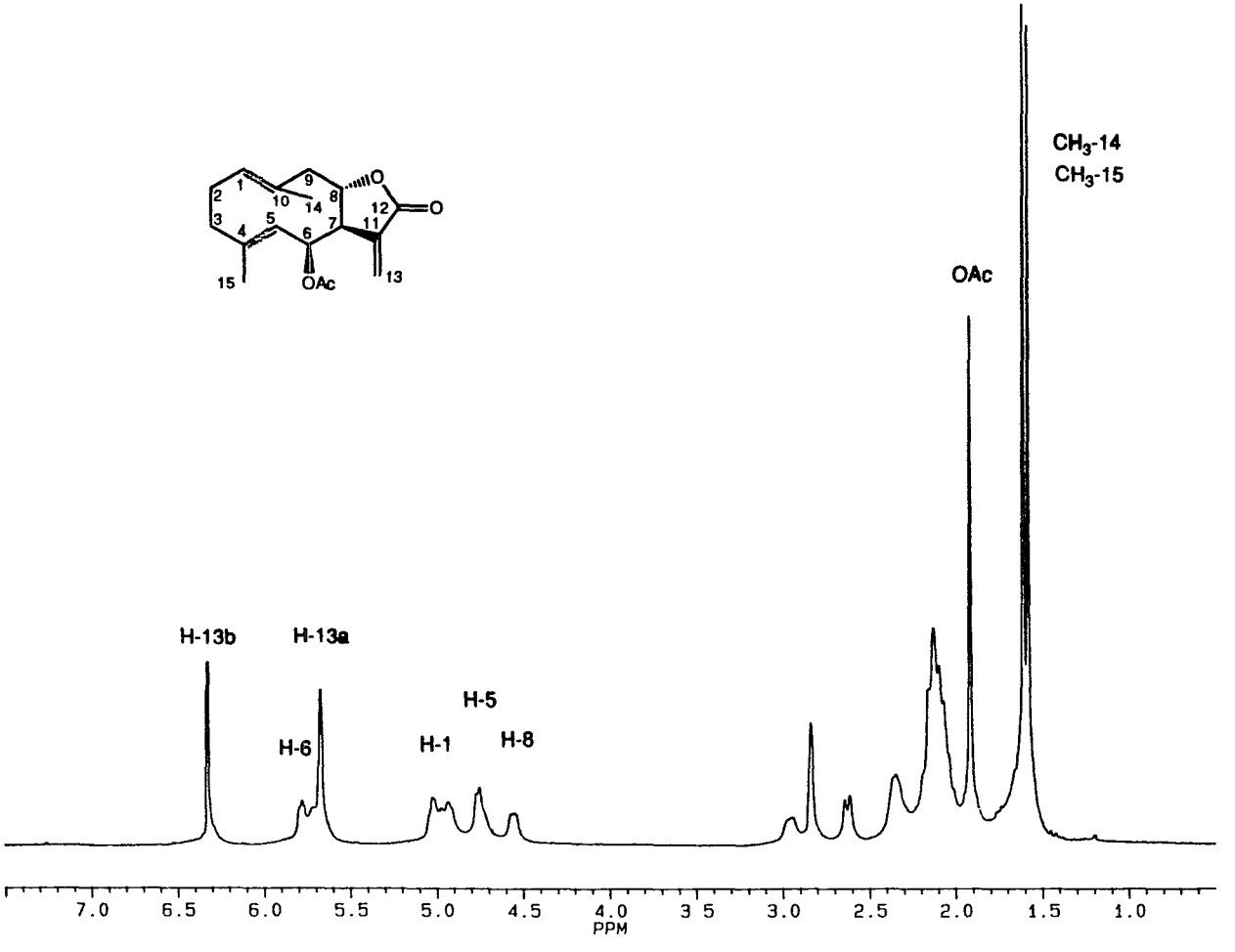


Figure 4.23. ^{1}H NMR spectrum of compound **41** in $CDCl_3$.

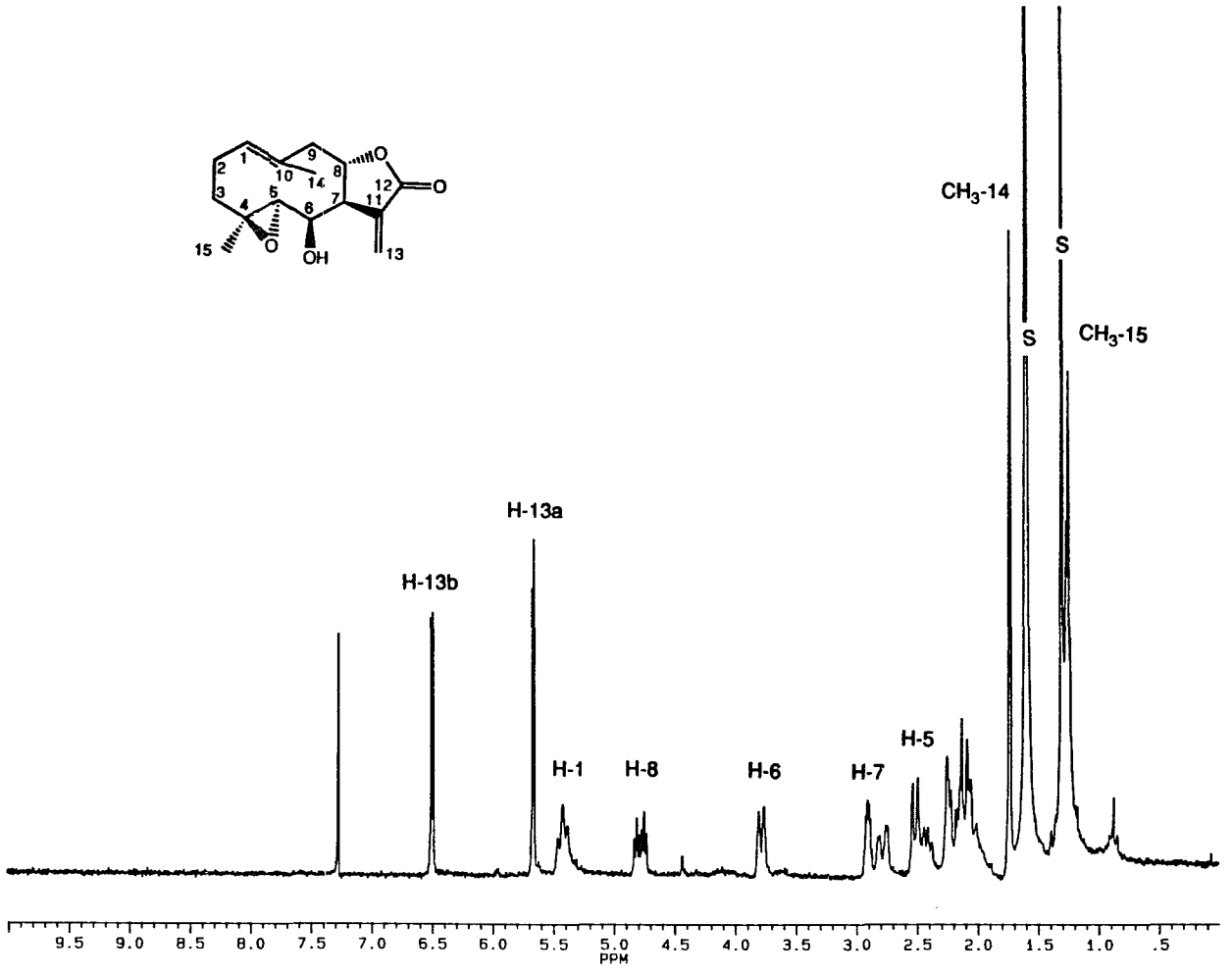


Figure 4.24. ^{1}H NMR spectrum of compound **42** in $CDCl_3$.

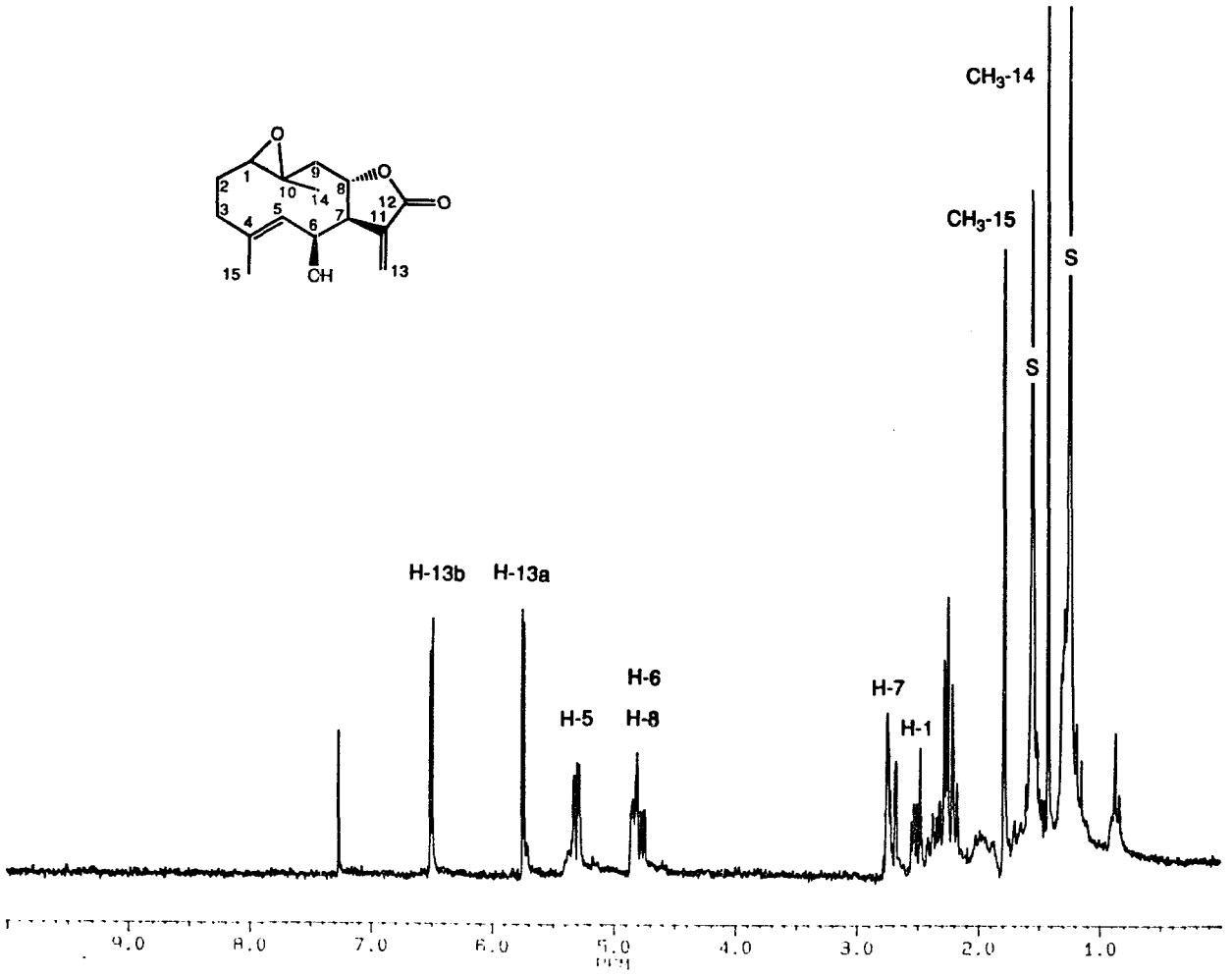


Figure 4.25. ^{1}H NMR spectrum of compound **43** in $CDCl_3$.

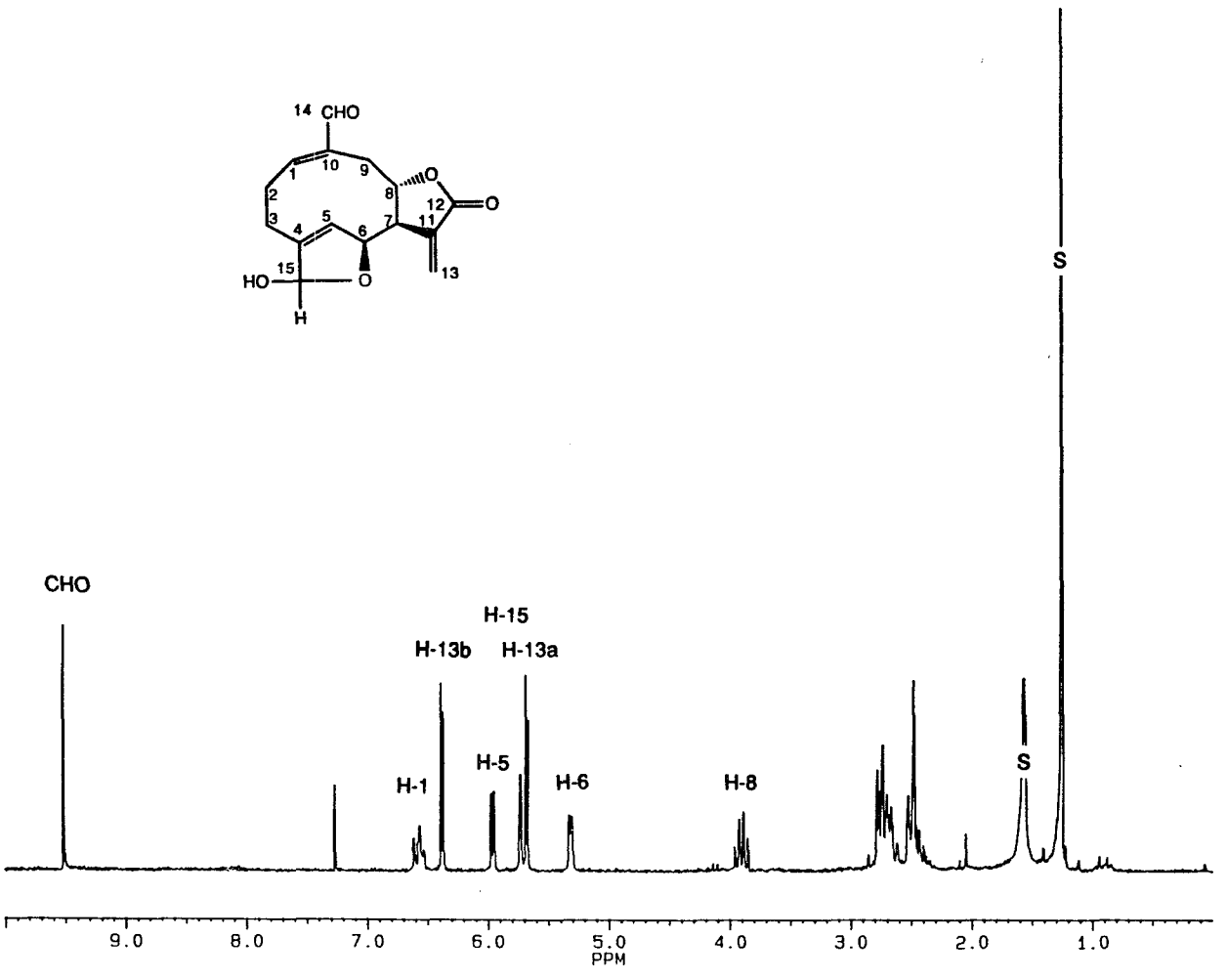


Figure 4.26. ^{1}H NMR spectrum of compound **44** in $CDCl_3$.

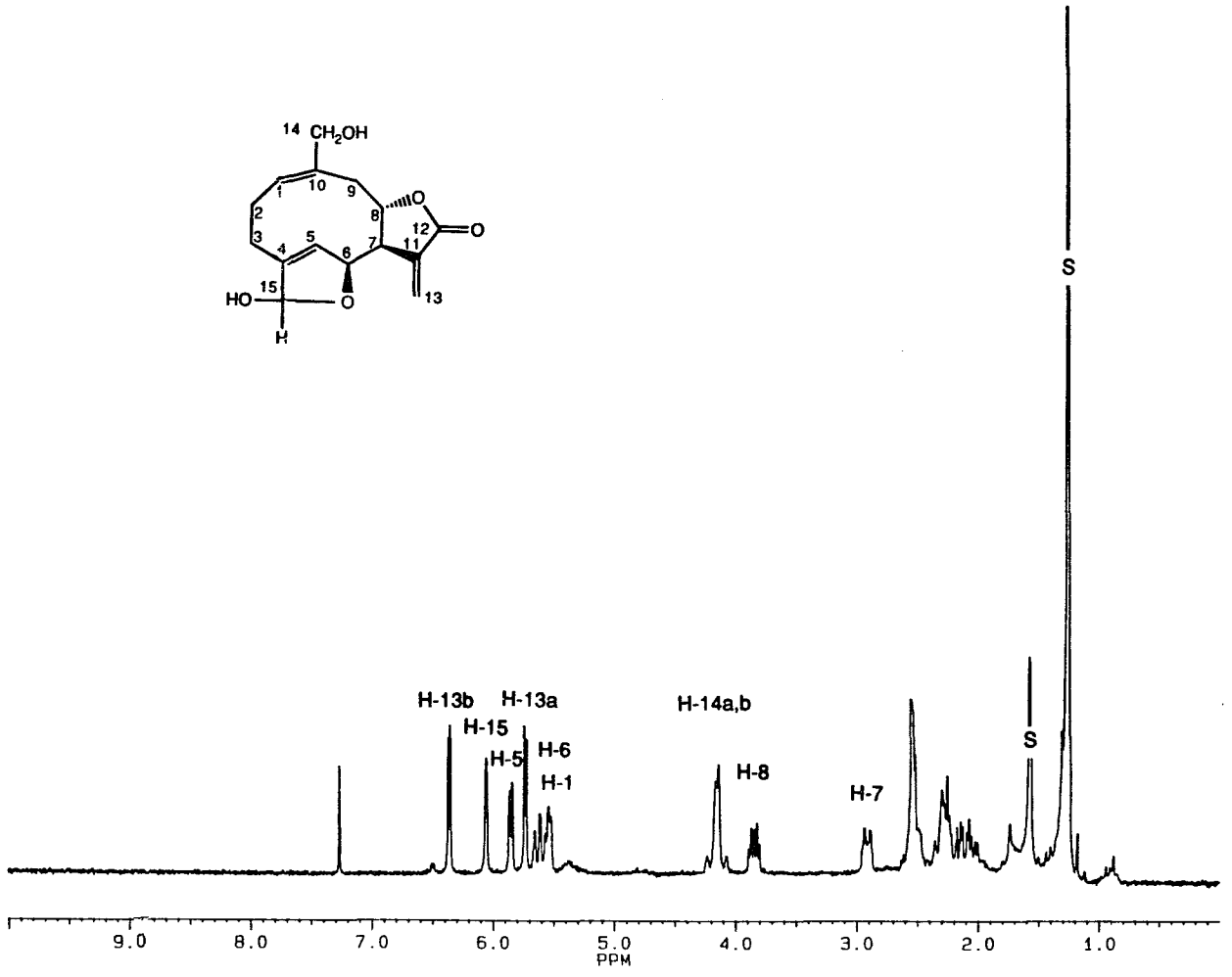


Figure 4.27. ^{1}H NMR spectrum of compound **45** in $CDCl_3$.

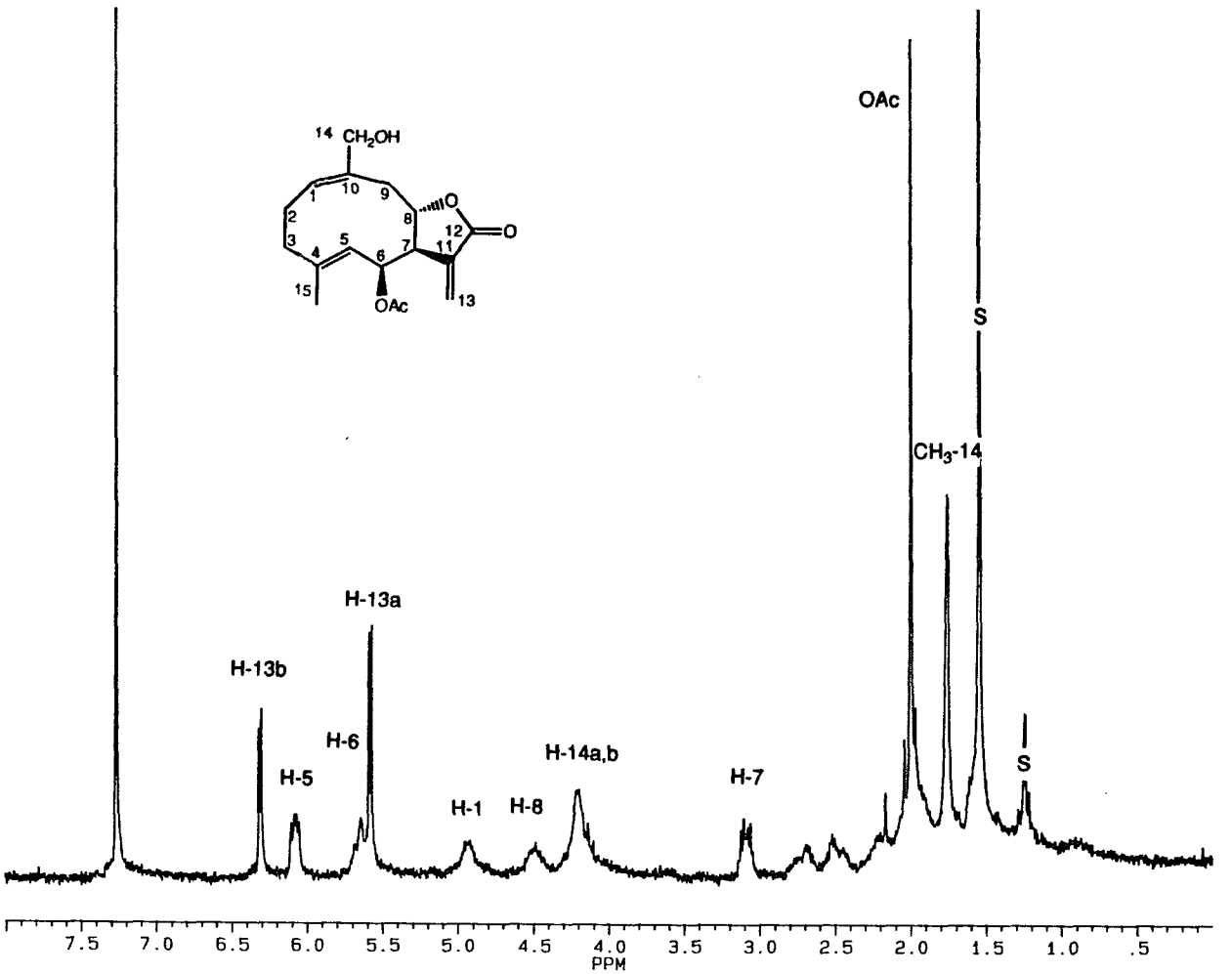


Figure 4.28. ^{1}H NMR spectrum of compound **46** in $CDCl_3$.

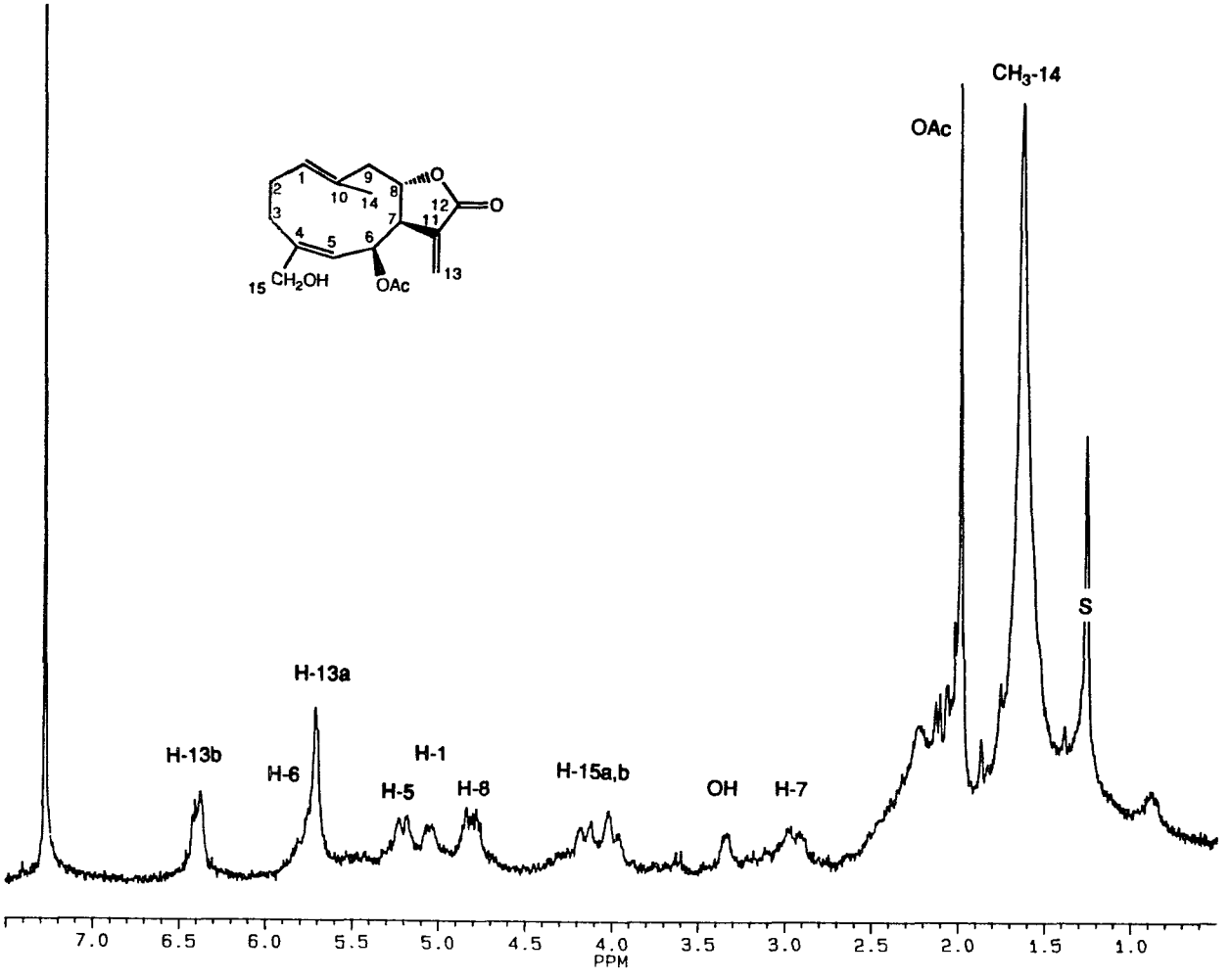


Figure 4.29. ^{1}H NMR spectrum of compound **47** in $CDCl_3$.

References

1. Fischer,N.H.; Olivier, E.J.; Fischer, H.D. "The Biogenesis and Chemistry of Sesquiterpene Lactones" in *Prog. Chem. Org. Nat. Prod.*, W.Herz, H. Grisebach, G.W. Kirby, eds. 1979, Springer, Wien, New York, Vol. 38, pp.47-390.
2. Rogers, D.; Moss, G. P.; Neidle, S. *Chem. Comm.* **1972**, 142-3.
3. Fischer, N.H. "Sesquiterpene Lactones: Biogenesis and Biomimetic Transformations." in Recent Advances in Phytochemistry, G. H. N. Towers, H. A. Stafford, eds., Plenum, New York, Vol. 24, pp.161-201.
4. Krishnan, S.; Paknikar, S.K.; Bhattacharyya, S.C.; Hall, A.L.; Herz, W. *J. Indian Chem. Soc.* **1978**, *55*, 1142-7.
5. Haruna, M.; Ito, K. *J. Chem. Soc. Chem. Comm.* **1981**, 483-5.
6. El-Feraly, F. S. *Phytochemistry* **1984**, *23*, 2372-4.
7. Malcolm, A.J. Ph.D. dissertation, Louisiana State University, Baton Rouge, Louisiana, p. 215.
8. Rodrigues, A.A.S.; Garcia, M.; Rabi, J.A. *Phytochemistry* **1978**, *17*, 953-4.
9. Umbreit, M.A.; Sharpless, K.B. *J. Am. Chem. Soc.* **1977**, *99*, 5526-8.
10. Coll, J.C.; Bowden, B.F. *J. Nat. Prod.* **1986**, *49*, 934-6.
11. Yoshioka, H.; Renold, W.; Fischer, N.H.; Higo, A.; Mabry, T.J. *Phytochemistry* **1970**, *9*, 823-32.
12. Yoshioka, H.; Mabry, T.J.; Timmerman, B.N. Sesquiterpene Lactones: Chemistry, NMR, and Plant Distribution. **1973**, University of Tokyo Press.
13. Jerussi, R.A. Selective Organic Transformations **1970**, Vol. 1, B.S. Thyagarajan, ed., Wiley, New York, N.Y., p. 301.

14. Sharpless, K.B.; Katsuki, T. *J. Am. Chem. Soc.* **1980**, *102*, 5974-6.

15. Quijano, L.; Calderon, J.S.; Gomez, G.F.; Lopez, P.J.; Rios, T.; Fronczek, F.R. *Phytochemistry* **1984**, *23*, 1971-4.

16. Chhabra, B.R.; Hayano, K. *Chem. Lett.* **1981**, 1703-6.

17. Bohlmann, F.; Schmeda-Hirschmann, J.; Jakupovic. *Phytochemistry* **1984**, *23*, 1435-7.

18. Samek, Z. *Collect. Czech. Chem. Commun.* **1978**, *43*, 3210-26.

19. Herz, W.; Wahlberg, I. *J. Org. Chem.* **1973**, *38*, 2485-9.

20. Loev, B.; Goodman, M.M. *Chem. and Ind.* **1967**, 2026-32.

Chapter 5. Miscellaneous Modifications of Sesquiterpene Lactones

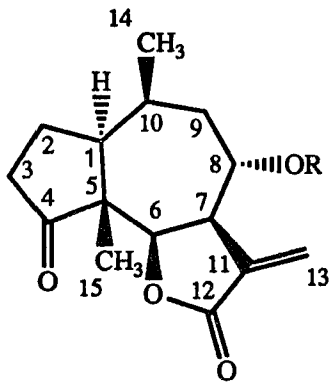
Part. A. Catalytic Hydrogenation of Confertiflorin

Introduction

Both catalytic hydrogenation[1,2] and sodium borohydride[3] ($NaBH_4$) have been used to reduce the exocyclic methylene moiety of α-methylene-γ-lactones to their respective 11,13-dihydroderivatives. Since $NaBH_4$ also easily reduces ketones or aldehydes to alcohols[4], this is not the reagent of choice for exocyclic methylene reduction of sesquiterpene lactones which contain ketone or aldehyde moieties. Since it was desired to selectively reduce the exocyclic methylene group of confertiflorin (**1**), catalytic hydrogenation was used.

Results and Discussion

The catalytic hydrogenation of confertiflorin (**1**) was carried out in methanol using a 10% Pd-charcoal catalyst as descibed previously in the literature.[5] Two major products were isolated: isoconfertiflorin (**3**) (44%) and dihydroconfertiflorin (**5**) (34%) (Scheme 5.1). The ^1H NMR data for these two compounds is identical to that reported previously.[5] A minor product, desacetylisoconfertiflorin (**4**) (13%) was also isolated and probably results from the hydrogenolysis of isoconfertiflorin (**3**). Compound **4** was not isolated form the catalytic hydrogenation of compound **1** previously, however, compound **4** was the major product isolated from the catalytic hydrogenation of desacetylconfertiflorin (**2**). All three products were analyzed by ^1H and ^{13}C NMR, FTIR, and mass spectral data. The ^{13}C NMR assignments for compounds **1, 3-5** appear in Table 5.1 and were made by comparison with the ^{13}C NMR assignments of similar compounds.[6] The X-ray crystal structure of isoconfertiflorin (**3**) was also solved.



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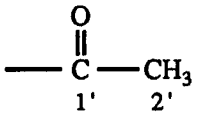
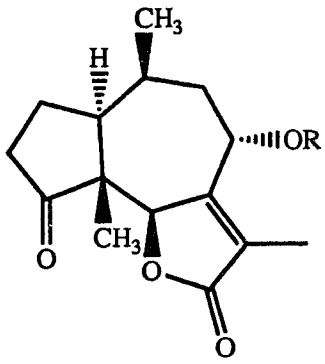
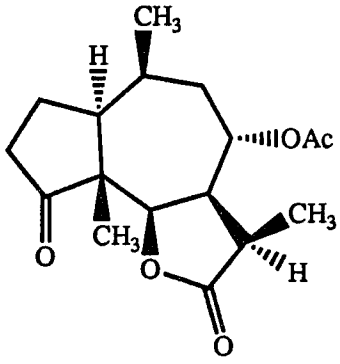
2 R = H

Ac = 

H_2
Pd/C



3 R = Ac
4 R = H



5

Scheme 5.1

Table 5.1 ^{13}C NMR assignments for compounds **1, 3-5**.[a]

Carbon atom	1	3	4	5
1	48.4	43.0	42.6	47.9
2	23.4	23.7	23.9	22.9
3	39.8	37.4	38.6	37.2
4	217.6	217.1	219.0	216.4
5	54.4	52.5	52.6	53.7
6	68.8	66.9	65.0	68.2
7	45.5	155.8	161.9	46.5
8	79.8	82.9	83.3	82.0
9	31.1	29.5	29.3	31.1
10	35.6	36.3	37.7	35.6
11	136.1	127.2	124.5	40.8
12	169.5	173.1[b]	174.7	178.6
13	123.0	17.8	17.9	12.2
14	15.7	10.4	10.4	13.8
15	13.5	8.7	8.6	14.8
C-1'	169.5	169.5[b]	---	169.8
C-2'	21.0	21.1	---	21.2

a = Spectra were recorded at 200MHz in $CDCl_3$ with Me_4Si as internal standard.
b = interchangeable signals

Experimental Section

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker-AC-200 spectrometer in $CDCl_3$ using $SiMe_4$ as an internal standard. Mass spectra were recorded on a HP5985 spectrometer. Infrared spectra were recorded on a Perkin-Elmer 1760x spectrometer in film on NaCl plates.

Chromatographic separations were made on silica gel (60-200M, J. T. Baker Chemical Co.).

X-ray intensity data were collected by ω-2θ scans on an Enraf-Nonius CAD4 diffractometer equipped with CuKα radiation (λ = 1.54184Å) and a graphite monochromator. One hemisphere of data was collected within $2° < \theta < 75°$. The structure was solved using direct methods and refined by full-matrix least squares using the Enraf-Nonius SDP. Nonhydrogen atoms were refined anisotropically. Hydrogen atoms were located by difference maps; those of the acetate group were allowed to ride on the carbon atoms to which they are bonded, with refined isotropic thermal parameters, while all other hydrogen atoms were refined isotropically.

A sample of confertiflorin (**1**) had been previously isolated from *Ambrosia confertiflora*. **Conferiflorin (1)** IR 1761, 1735, 1656cm^{-1}; ^1H NMR (Fig. 5.1): δ 6.23 (d,1H,C_{13}-Hb,J=3Hz), 5.58 (d,1H,C_{13}-Ha,J=3Hz), 5.30 (ddd,1H,C_8-H,J=9,9,3Hz), 4.57 (d,1H,C_6-H,J=9Hz), 3.46 (m,1H,C_7-H), 2.03 (s,3H,OAc), 1.13 (d,3H,C_{14}-CH3,J=7Hz), 1.02 (s,3H,C_{15}-CH_3); MS *m/z* (relative intensity) 306 (M^+) (0.5), 247 (M-59$^+$) (2.8), 246 (M-60$^+$) (10.7), 231 (M-75$^+$) (100).

Catalytic hydrogenation of confertiflorin (1). Confertiflorin (**1**) (190mg) was dissolved in 10ml of methanol and 20mg of 10% Pd-charcoal catalyst was added. The solution was catalytically hydrogenated for 3hrs. After this time, the solution was filtered to remove the catalyst and the solvent was evaporated. The

products were separated by silica gel column chromatography eluting with 60/40 hexane/ethyl acetate and gradually increasing the polarity of the mobile phase. Isoconfertiflorin (**3**) (80mg, 44%) eluted in the early fractions followed by dihydroconfertiflorin (**5**) (62mg, 34%), followed by desacetylisoconfertiflorin (**4**) (20mg, 13%).

Isoconfertiflorin (**3**) IR 1752, 1735cm^{-1}; ^{1}H NMR (Fig. 5.2): δ 5.97 (dd,1H,C_8-H,J=6,1Hz), 4.73 (d,1H,C_6-H,J=2Hz), 2.05 (s,3H,OAc), 1.87 (d,3H,C_{13}-CH_3,J=2Hz), 1.01 (d,3H,C_{14}-CH_3,J=7Hz), 0.80 (s,3H,C_{15}-CH_3); MS *m/z* (relative intensity) 306 (M^+) (0.1), 246 (M-60$^+$) (2.9). The molecular structure of isoconfertiflorin (**3**) is shown in Figure 5.5. Crystal data for **3**: $C_{17}H_{22}O_5$, MW = 306.4, trigonal space group $P3_1$, a = 10.4498(5), c = 12.7516(8) Å, Z = 3, Dc = 1.266gcm^{-3}, R = 0.026 for 1666 observed data.

Desacetylisoconfertiflorin (**4**) IR 3475, 1743cm^{-1}; ^{1}H NMR (Fig. 5.3): δ 5.13 (m,1H,C_8-H), 4.96 (d,1H,C_6-H,J=2Hz), 1.81 (d,3H,C_{13}-CH_3,J=2Hz), 1.00 (d,3H,C_{14}-CH_3,J=7Hz), 0.75 (s,3H,C_{15}-CH_3); MS *m/z* (relative intensity) 264 (M^+) (9.9), 249 (M-15$^+$) (10.5), 246 (M-18$^+$) (36.8), 231 (M-33$^+$) (35.2).

Dihydroconfertiflorin (**5**) IR 1770, 1735cm^{-1}; ^{1}H NMR (Fig. 5.4): δ 5.23 (ddd,1H,C_8-H,J=10,8,4Hz), 4.65 (d,1H,C_6-H,J=9Hz), 3.15 (dd,1H,C_7-H,J=10Hz), 2.03 (s,3H,OAc), 1.21 (d,3H,C_{13}-CH_3,J=8Hz), 1.21 (d,3H,C_{14}-CH_3,J=8Hz), 1.09 (s,3H,C_{15}-CH_3); MS *m/z* (relative intensity) 308 (M^+) (1.3), 293 (M-15$^+$) (1.2), 249 (M-59$^+$) (2.9).

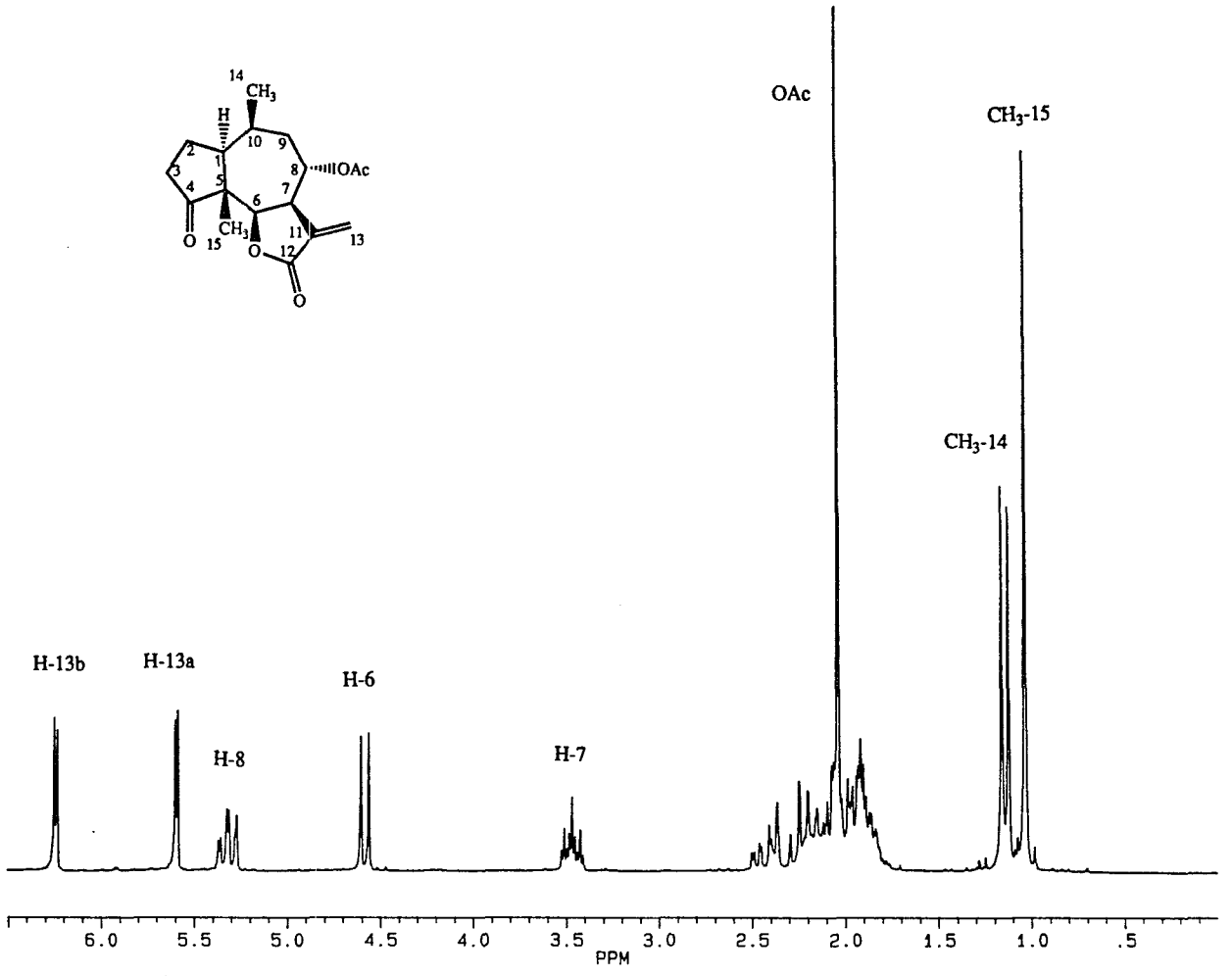


Figure 5.1. ^{1}H NMR spectrum of confertiflorin (1) in $CDCl_3$.

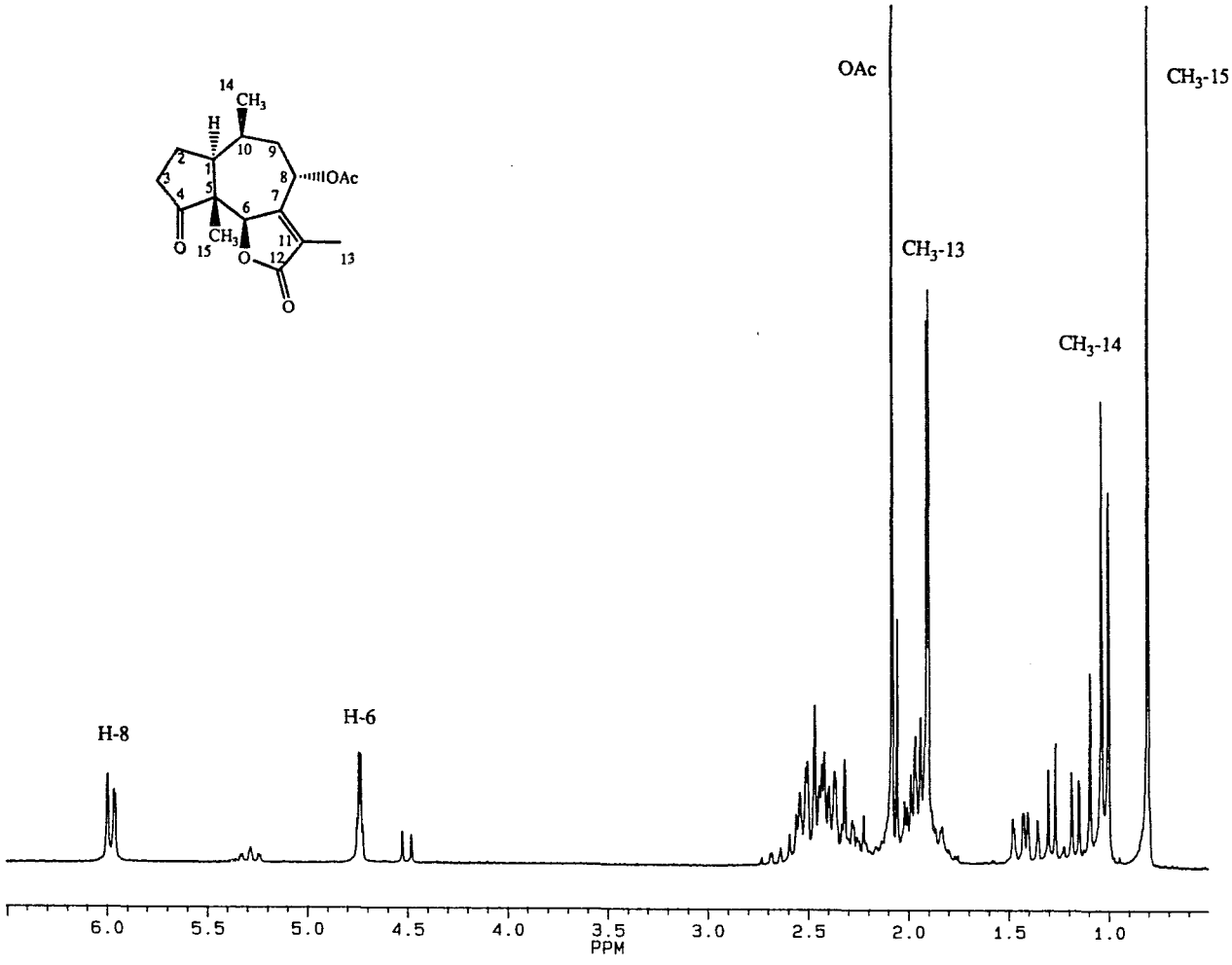


Figure 5.2. ^{1}H NMR spectrum of isoconfertiflorin (3) in $CDCl_3$.

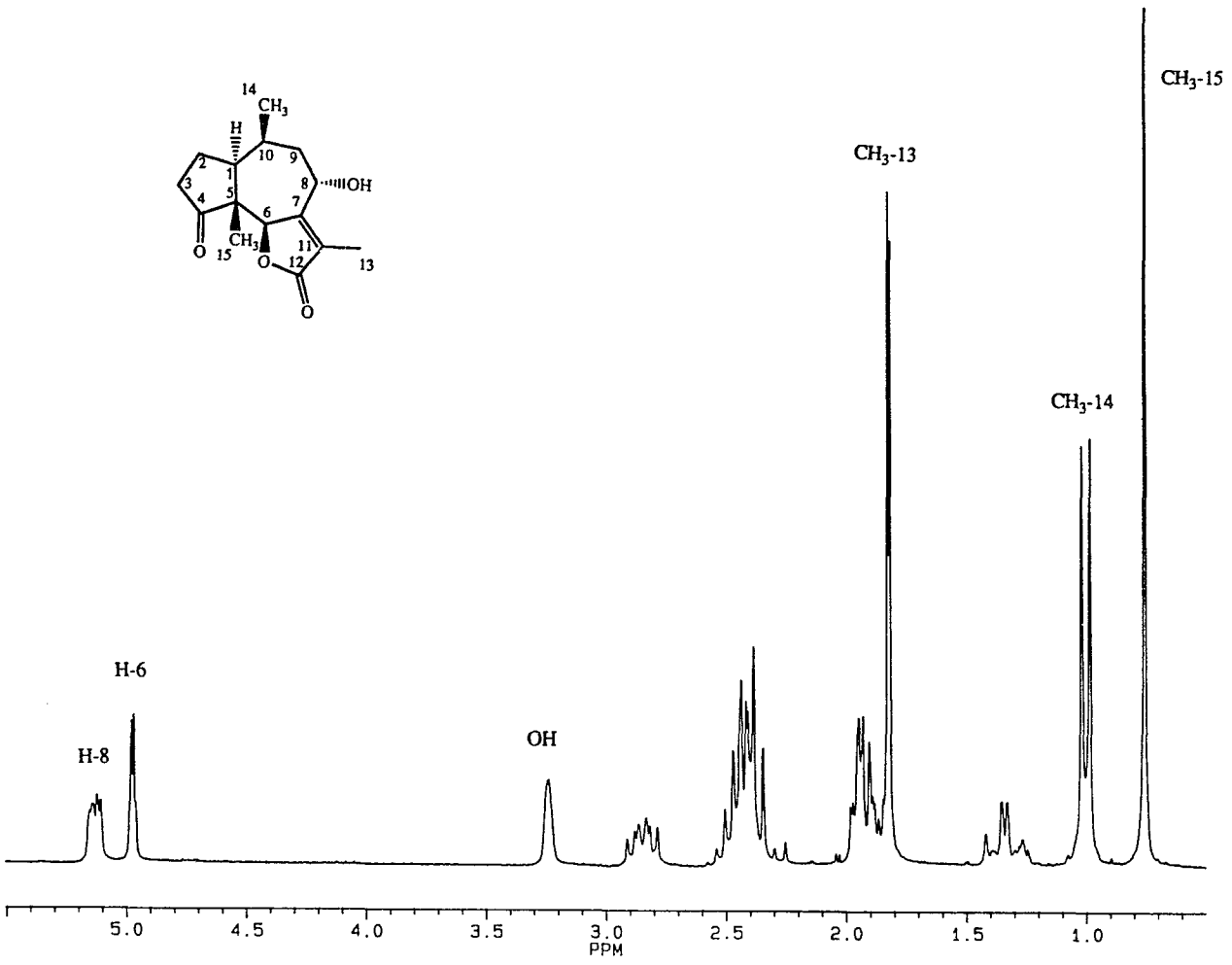


Figure 5.3. ^{1}H NMR spectrum of desacetylisoconfertiflorin (4) in $CDCl_3$.

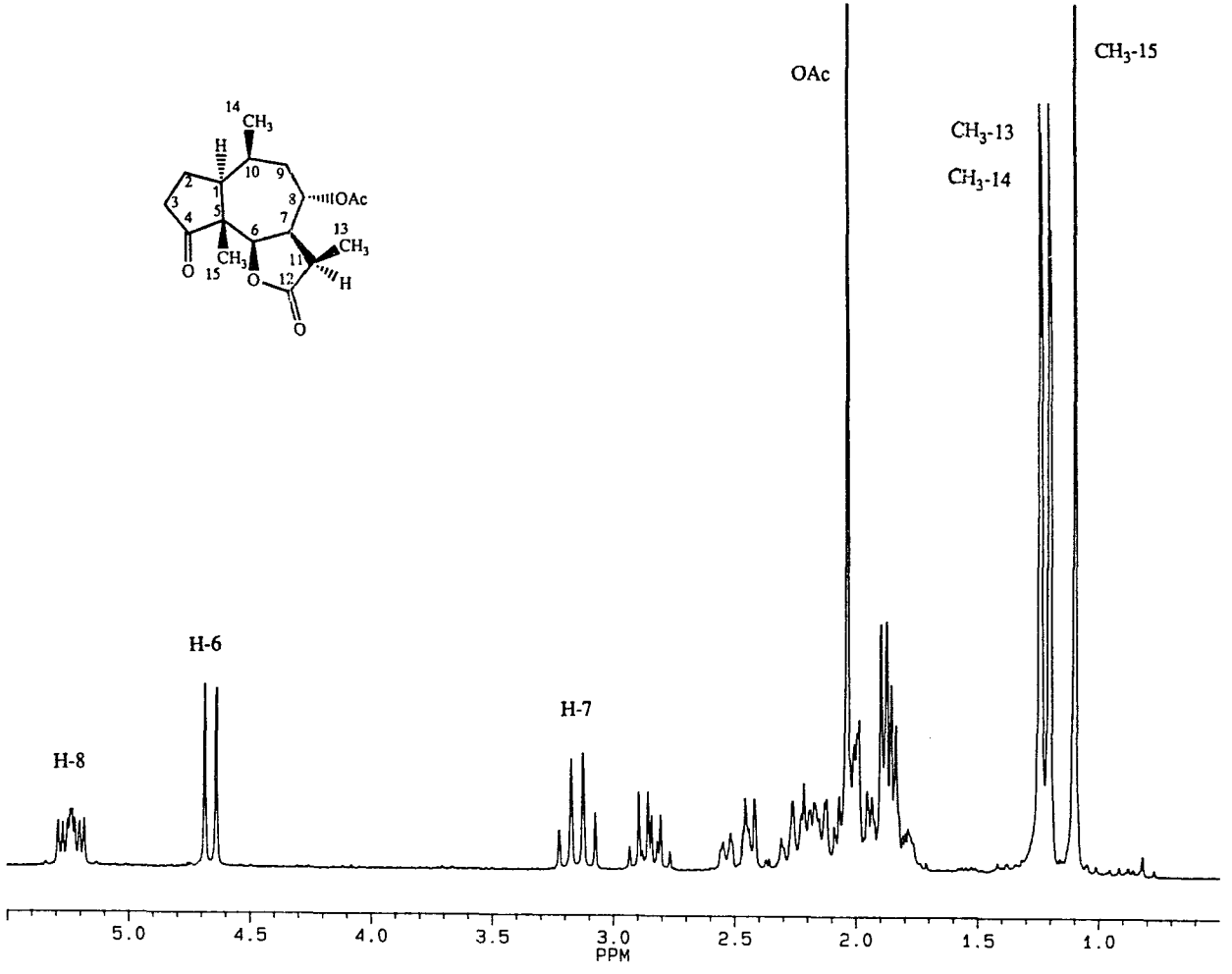


Figure 5.4. ^{1}H NMR spectrum of dihydroconfertiflorin (5) in $CDCl_3$.

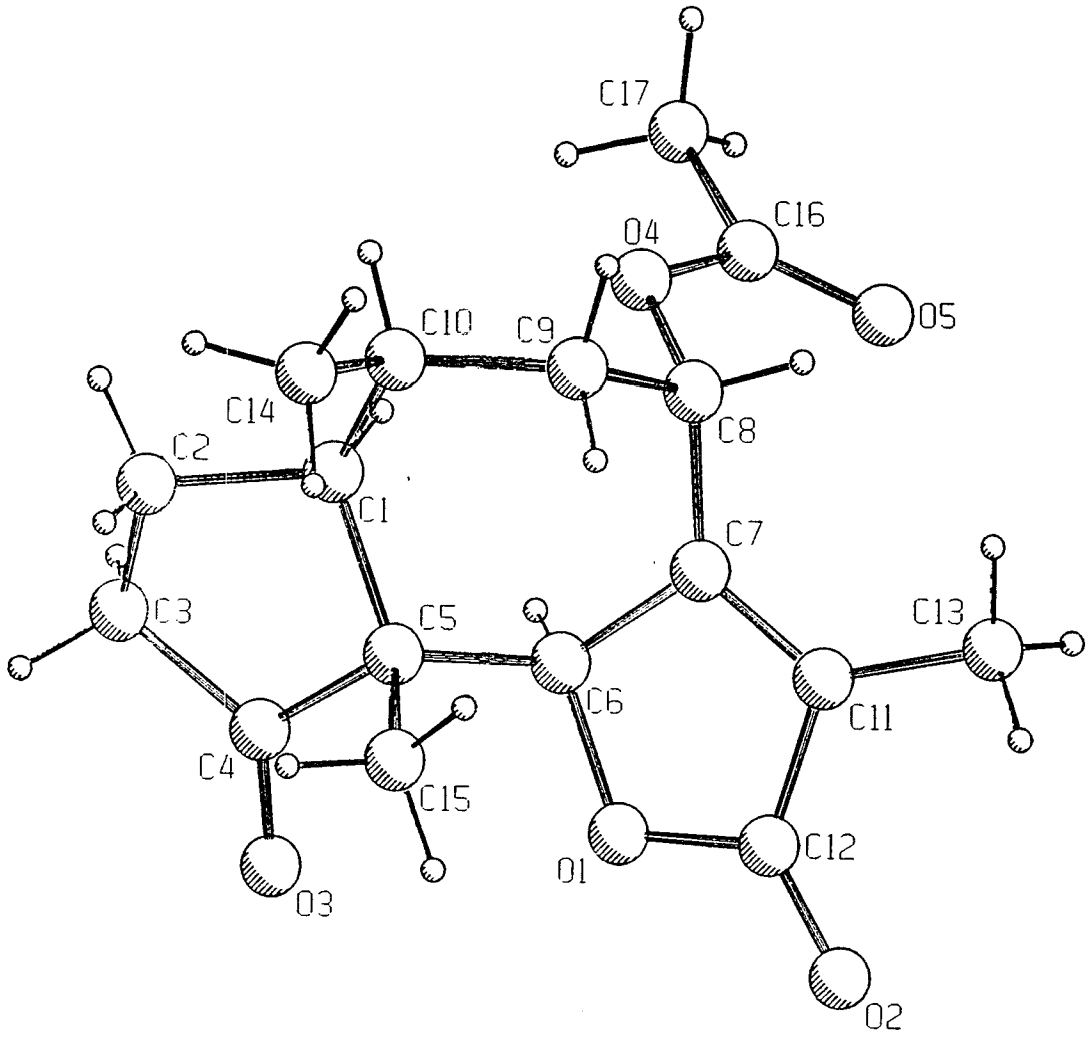


Figure 5.5. Molecular structure of isoconfertiflorin (3).

References

1. Fischer, N. H.; Mabry, T.J.; Kagan, H. B. *Tetrahedron* **1968**, *24*, 4091-97.
2. Fischer, N. H.; Mabry, T. J. *Chem. Commun.* **1967**, 1235-36.
3. Renold, W.; Yoshioka, H.; Mabry, T. J. *J. Org. Chem.* **1970**, *35*, 4264-6.
4. Augustine, R. L. (ed.) Reductive Techniques in Organic Synthesis. **1968**, Marcel Dekker, New York, pp. 19-40.
5. Fischer, N. H.; Mabry, T. J. *Tetrahedron* **1967**, *23*, 2529-38.
6. Vargas, D.; Fronczek, F. R.; Fischer, N. H.; Hostettmann, K. *J. Nat. Prod.* **1986**, *49*, 133-8.

Part B. Attempted allylic oxidations of germacrolides with chromium trioxide-pyridine complex and with tert-butyl hydroperoxide in the presence of a chromium hexacarbonyl catalyst.

Introduction

Many germacranolides contain oxygen functionalities at C-2, C-3, and C-9 (Scheme 5.2) indicating biogenetic allylic oxidations with methylene preference over methyl groups. Most *in vitro* biomimetic allylic oxidations of germacrolides have been carried out with tert-butyl hydroperoxide (tBuOOH) in the presence of selenium dioxide[2] (SeO_2) and result in specific allylic methyl group oxidation. The opposite regioselectivity has not been observed.

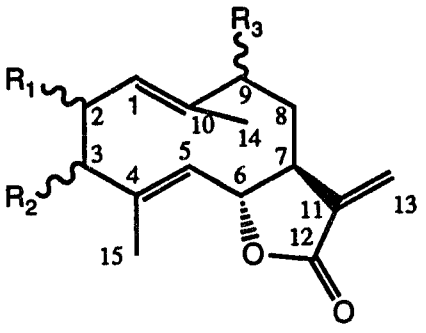
Attempts were made to carry out a regiospecific allylic methylene oxidation (at C-2 or C-9) of a germacrolide (dihydroparthenolide, Scheme 5.3) using chromium trioxide-pyridine complex and tBuOOH in the presence of a chromium carbonyl catalyst.

Dauben et al.[3] reported that the chromium trioxide-pyridine oxidation of alkenes to α,β-unsaturated ketones occurs with very little allylic oxidation at methyl groups. This selectivity (methylene > methyl) has been rationalized mechanistically as resulting from an initial hydrogen atom (or hydride) abstraction from the alkene to form an allylic radical (or carbocation) (Scheme 5.4). The resulting species is then oxidized at either end of the allylic radical (or carbocation) yielding an α,β-unsaturated ketone.

Pearson et al.[4] observed similar selectivities when alkenes were treated with tBuOOH in the presence of $Cr(CO)_6$ or $Cr(CO)_x(CH_3CN)_y$ species. The mechanism of this reaction has yet to be explored.

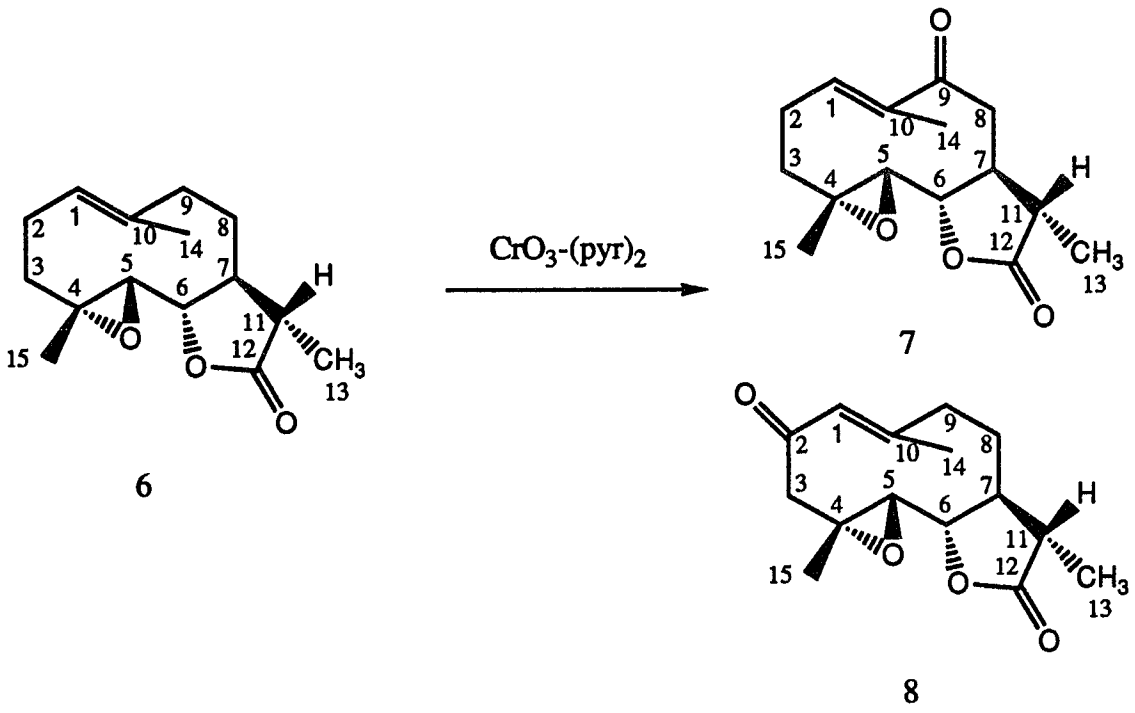
Results and Discussion

An attempt was made to carry out the allylic oxidation of dihydroparthenolide

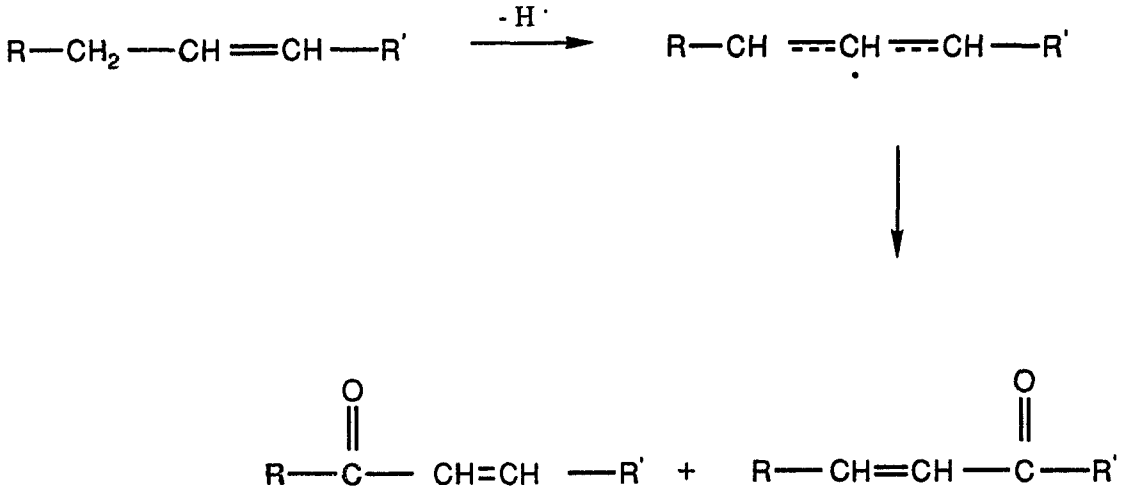


R_1 = α-OH, R_2 = R_3 = H	Tamaulipin A
R_1 = H, R_2 = α-OH, R_3 =H	Tamaulipin B
R_1 = R_2 = H, R_3 = β-OH	Haageanolide
R_1 = H, R_2 = α-OH, R_3 = OH	Salonitenolide

Scheme 5.2



Scheme 5.3



Scheme 5.4

(DHP, **6**) at C-2 or C-9 using the improved procedure for the preparation of the chromium trioxide-pyridine complex developed by Ratcliffe and Rodenhorst.[5] In this procedure, the brick-red complex, CrO_3-$(pyr)_2$ is not isolated but is used as prepared in a dichloromethane solution.

After standard work-up procedures,[3,5] the ^1H NMR spectrum of the crude product mixture showed the presence of unreacted DHP (**6**,65%) and the diepoxide (**9**, 35%) (Scheme 5.4), 1,10-epoxydihydroparthenolide. Diepoxide **9** was separated from DHP (**6**) by silica gel column chromatography and was shown to be identical to the meta-chloroperoxybenzoic acid (mCPBA) epoxidation product of DHP (**6**). No allylic oxidation products were isolated.

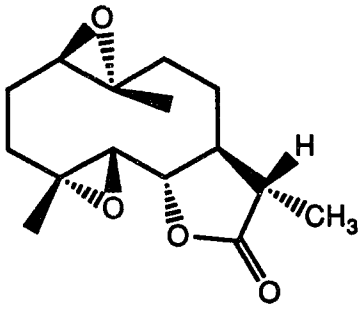
Apparently, the oxidation potential of the chromium trioxide-pyridine complex is sufficient to effect electron transfer from the olefin (**6**) leading to formation of an epoxide (**9**) analogous to the reaction of chromyl chloride with olefins[6] (Scheme 5.6).

Allylic oxidation of DHP (**6**) was also attempted using tBuOOH in the presence of $Cr(CO)_x(CH_3CN)_y$ as a catalyst. The major product from this reaction was 1,10-epoxydihydroparthenolide (**9**). Some unreacted DHP (**6**) was also recovered. Again, no allylic oxidation products were isolated.

Experimental Section

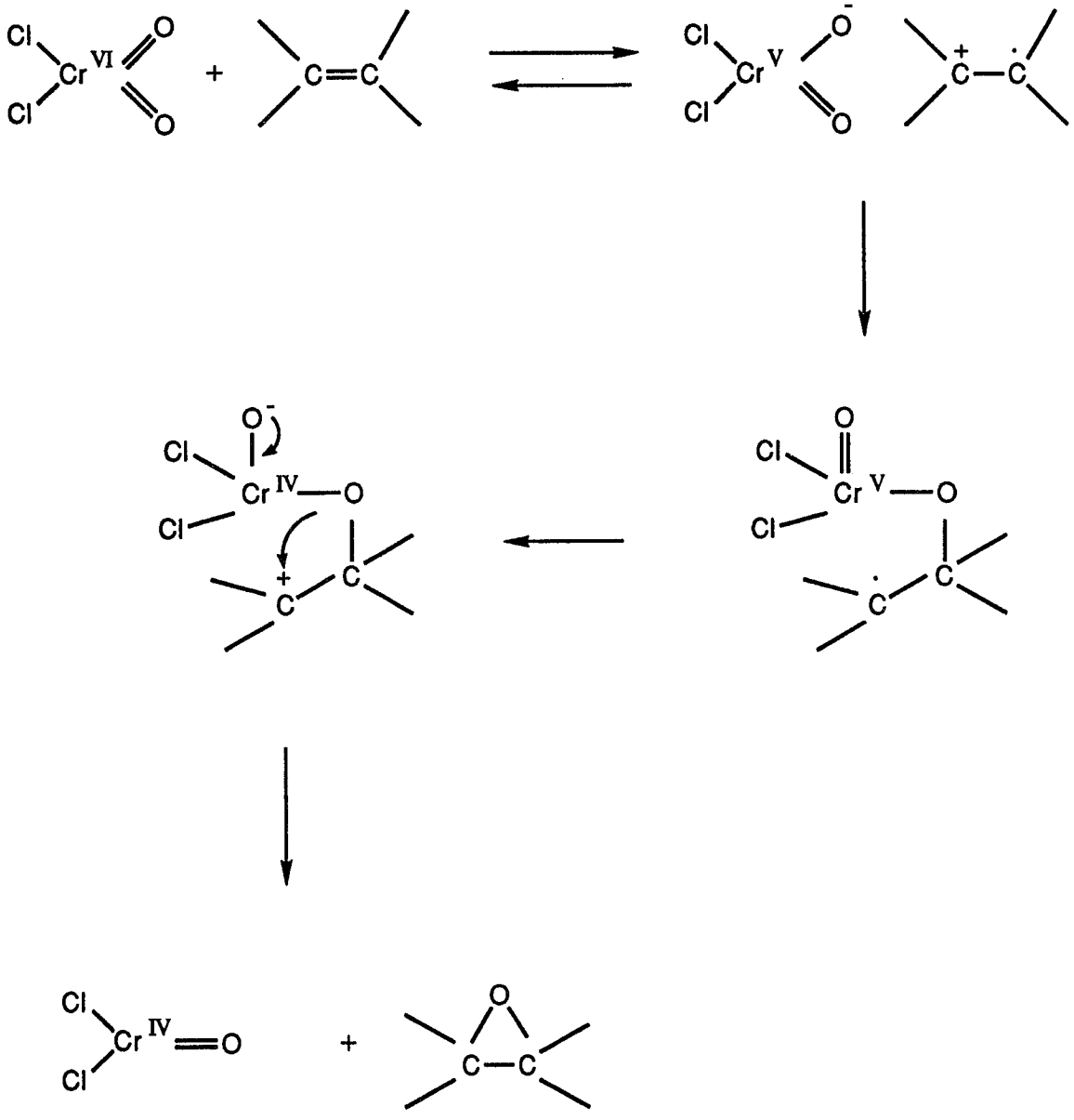
Dihydroparthenolide (**6**) was isolated from the aerial parts of *Ambrosia artemisiifolia*.[7,8]

Preparation of the chromium trioxide-pyridine complex in DCM solution.[4] Chromium trioxide (1.2g) was dried in a vacuum dessicator over P_2O_5 for 24hrs. Distilled pyridine (2ml), which was stored over 4Å molecular sieves,



9

Scheme 5.5



Scheme 5.6

was added to 15ml of DCM in a round-bottom flask equipped with a drying tube. The solution was stirred at room temp. and the CrO_3 was then added. The solution turned from yellow to deep burgundy in 2 minutes.

Attempted allylic oxidation of DHP (6) with chromium trioxide-pyridine complex. After stirring the DCM solution of the oxidant for an additional 15 minutes, a DCM solution of DHP (200mg) was added by syringe. The reaction mixture was stirred at room temp. for 24hrs. After this time, the solution was filtered and the filtrate was washed with saturated aqueous $NaHCO_3$, dried over anhydrous Na_2SO_4, and the solvent was evaporated. The residue was subjected to silica gel column chromatography from which unreacted DHP (**6**) and 1,10-epoxydihydroparthenolide (**9**) were isolated.

Attempted allylic oxidation of DHP (6) with tBuOOH in the presence of $Cr(CO)_x(CH_3CN)_y$. To a suspension of DHP (200mg, 0.8mmol) and $Cr(CO)_6$ (88mg, 0.4mmol) in 10ml of acetonitrile was added 0.22ml of 70% tBuOOH. The reaction mixture was gently refluxed for 24hrs., cooled to room temp., and filtered. The filtrate was diluted with ether (100ml), washed with water (2 x 20ml) and saturated $NaHCO_3$ (2 x 15ml) and dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was subjected to preparative TLC (eluting with 75/25 hexane/ethyl acetate) yielding both unreacted DHP (**6**) and 1,10-epoxydihydroparthenolide (**9**).

References

1. Fischer, N.H.; Olivier, E.J.; Fischer, H.D. "The Biogenesis and Chemistry of Sesquiterpene Lactones." in *Prog. Chem. Org. Nat. Prod.*, W. Herz, H. Grisebach, G.W. Kirby, eds. **1979**, Springer, Wien, New York, Vol. 38, pp. 47-390.
2. Haruna, M.; Ito, K. *J. Chem. Soc. Chem. Comm.* **1981**, 483-5.
3. Dauben, W.G.; Lorber, M. Fullerton, D.S. *J. Org. Chem.* **1969**, *34*, 3587-92.
4. Pearson, A.J.; Chen, Y-S.; Hsu, S-Y.; Ray, T. *Tetrahedron Lett.* **1984**, *25*, 1235-8.
5. Ratcliffe, R.; Rodenhorst, R. *J. Org. Chem.* **1970**, *35*, 4000-2.
6. Sheldon, R.A.; Kochi, J. K. Metal-Catalyzed Oxidations of Organic Compounds. Academic Press, New York, N.Y., **1981**, pp. 168-71.
7. Parodi, F. Dissertation, "Structure Elucidation of Natural Products From Asteraceae Using Modern NMR Techniques and Biomimetic Transformations of 11,13-Dihydroparthenolide." Louisiana State University, **1988**.
8. Pentes, H. Fischer, N.H., unpublished results.

Part C. Attempted dehydrations of 11-hydroxygermacranolides

Introduction

7-Hydroxy-α-methylene-γ-lactones are molluscicidal compounds which may be effective PFK inhibitors.[1-3] 11-Hydroxysesquiterpene lactones can be converted to 7-hydroxy-α-methylene-γ-lactones by dehydration to form the endocyclic double bond, followed by epoxidation, and then reductive opening of the epoxide. The first step in this synthetic sequence (see Scheme 1.4, Chapter 1), the dehydration of 11-hydroxysesquiterpene lactones was attempted using thionyl chloride in pyridine and with hexamethylphosphoramide (HMPA).

Results and Discussion

Secondary and tertiary alcohols can be dehydrated by treatment with thionyl chloride ($SOCl_2$) in pyridine.[4,5] An attempt was made to dehydrate an epimeric mixture of tertiary alcohol 11-hydroxydihydrocostunolide (**10** and **11**) under these conditions. The dehydration of compounds **10** and **11** generated a complex mixture of products from which the desired endocyclic elimination product isocostunolide (**12**) was isolated in small amounts by column chromatography. ^1H NMR data for the column fractions showed no evidence (no doublets at 6.2 and 5.5ppm) for the formation of any exocyclic double bond dehydration product costunolide (**13**).[6]

Monson[7,8] reported the direct solvent catalyzed dehydration of alcohols in hexamethylphosphoramide (HMPA) at reflux with little rearrangement products. The disadvantages of this method are the high temperature required for dehydration and the need to separate dimethylamine from the products. An attempt was made to dehydrate 11β-hydroxydihydrocostunolide (**11**) under these conditions. After reflux at 220-240°C for 1hr., the solution turned black. TLC analysis of the ether

extract of this residue showed no evidence of any sesquiterpene lactone products. Apparently, the harsh conditions required for solvent catalyzed dehydration lead to decomposition.

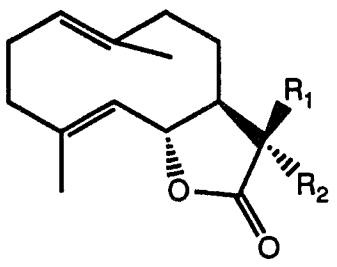
Experimental Section

^1H NMR spectra were recorded on a Bruker-AC200 spectrometer in $CDCl_3$ using $SiMe_4$ as an internal standard. Chromatographic separations were made on silica gel (60-200M, J. T. Baker Chemical Co.).

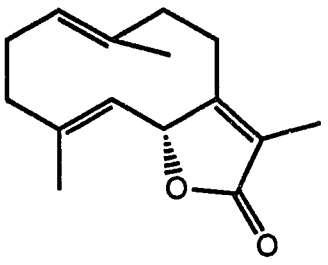
Dehydration of 11-hydroxydihydrocostunolide with $SOCl_2$ in pyridine. A mixture of 11α- and 11β-hydroxydihydrocostunolide (20mg) was dissolved in 5ml of pyridine. Excess $SOCl_2$ (1.5eq., 0.09ml) was added to the solution at room temp. The reaction solution was stirred for 3hrs. The solvent was evaporated and TLC analysis showed a complex mixture of products. Dry column (silica gel) chromatography was used to separate isocostunolide (**12**) (2-3mg) by eluting with mixtures of dichloromethane (DCM) and acetone.

Isocostunolide (12) ^1H NMR (Fig. 5.6): δ 5.87 (dd,1H,C_6-H,J=4Hz), 5.13 (d,1H,C_5-H,J=8Hz), 4.92 (m,1H,C_1-H), 1.83 (s,3H,C_{13}-CH_3), 1.56 (s,3H,C_{15}-CH_3), 1.15 (s,3H,C_{14}-CH_3).

Dehydration of 11β-hydroxydihydrocostunolide with HMPA. A solution of 16mg of 11β-hydroxydihydrocostunolide dissolved in 10ml of hexamethylphosphoramide (HMPA) was refluxed at 220-240°C. After 1hr., the solution changed from an initial yellow color to dark black. The reflux was stopped, the solution was cooled and then extracted with diethyl ether. The ether was evaporated and the residue was analyzed by TLC and ^1H NMR and showed no spots or signals indicative of a germacranolide skeleton.

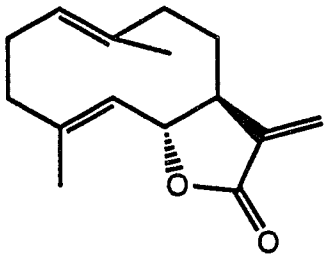


(i) or (ii)



10 $R_1 = CH_3$, $R_2 = OH$
11 $R_1 = OH$, $R_2 = CH_3$

12



13

(i) $SOCl_2$/pyridine

(ii) HMPA/reflux

Scheme 5.7

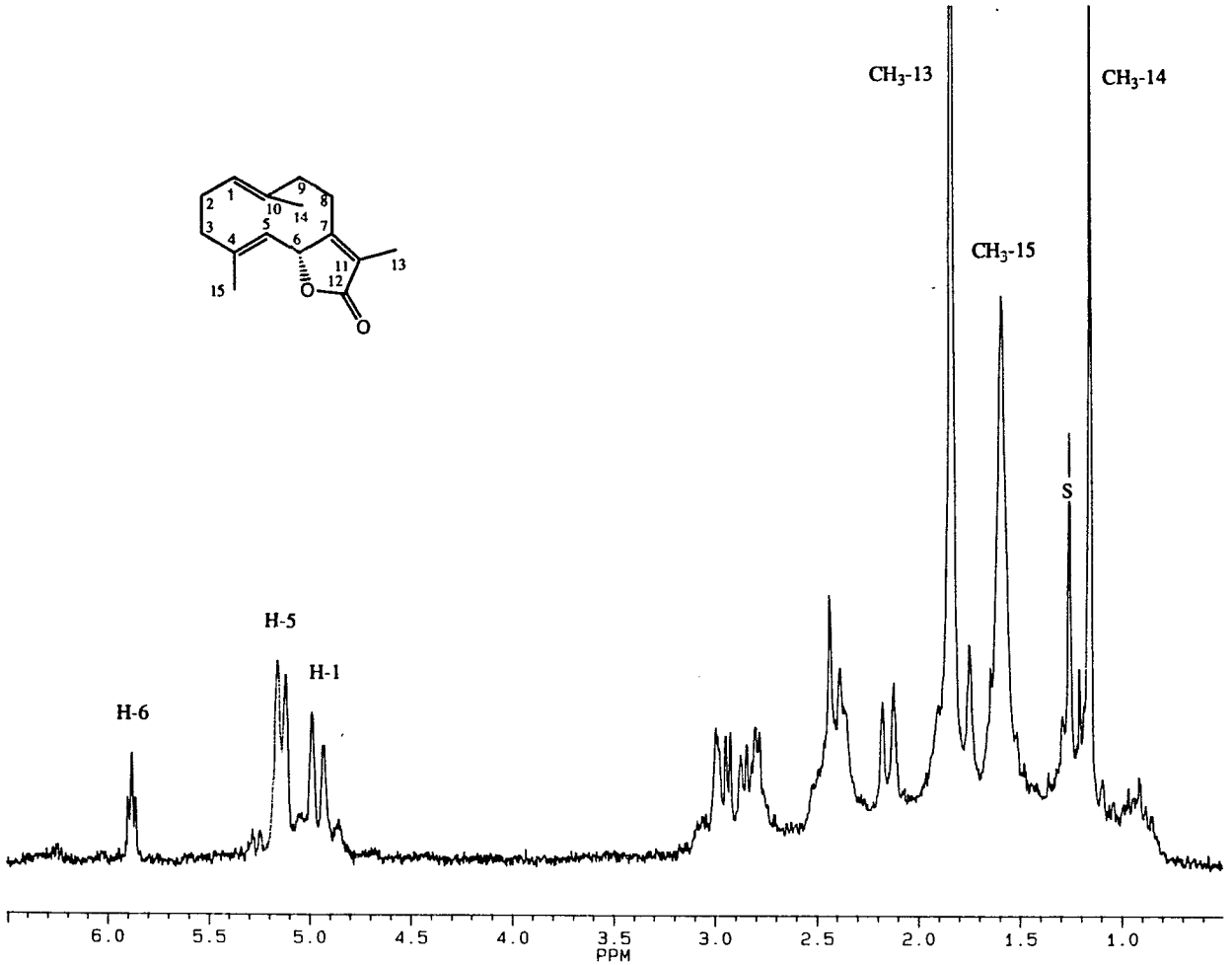


Figure 5.6. ^{1}H NMR spectrum of isocostunolide **(12)** in $CDCl_3$.

References

1. Fronczek, F.R.; Vargas, D.; Fischer, N.H.; Hostettmann, K. *J. Nat. Prod.* **1984**, *47*, 1036-9.
2. Vargas, D.; Fronczek, F.R.; Fischer, N.H.; Hostettmann, K. *J. Nat. Prod.* **1986**, *49*, 133-8.
3. Vargas, D.; Younathan, E.S.; Fischer, N.H., unpublished results.
4. Ruppert, J.F.; White, J.D. *J. Chem. Soc. Chem. Commun.* **1976**, 976-7.
5. Lomas, J.S.; Sagatys, D.S.; Dubois, J.E. *Tetrahedron Lett.* **1971**, *7*, 599-602.
6. Rodrigues, A.A.S.; Garcia, M.; Rabi, J.A. *Phytochemistry* **1978**, *17*, 953-4.
7. Monson, R.S. *Tetrahedron Lett.* **1971**, *7*, 567-70.
8. Monson, R.S.; Priest, D.N. *J. Org. Chem.* **1971**, *36*, 3826-8.

Part D. Reductive Opening of Epoxides with Aluminum Isopropoxide $Al(iOPr)_3$ and Lithium Diisopropylamide (LDA).

Introduction

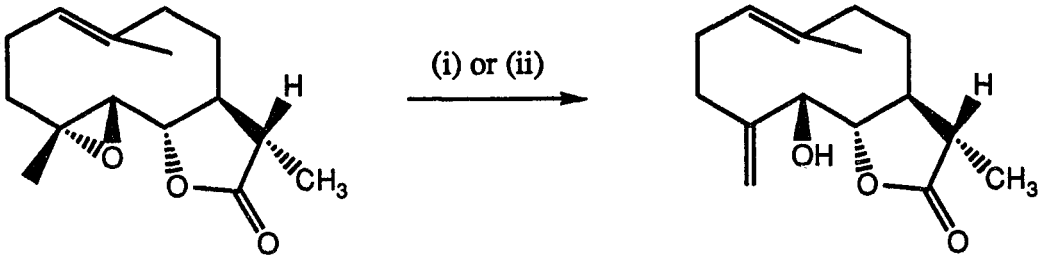
The biosynthesis of germacrolides like compound **15** (Scheme 5.8) is not known, however, compounds of this type may be derived from Hoffman-like reductive opening of their corresponding epoxides (**14**). Reductive opening of epoxides has been achieved using aluminum isopropoxide ($Al(iOPr)_3$) in refluxing toluene.[1-3] This methodology has been efficiently used on a eudesmanolide type sesquiterpene lactones.[4]

Base-promoted isomerizations with lithium diethylamide and lithium diisopropylamide (LDA) have also been used to convert epoxides to allylic alcohols.[5] Bellesia et al.[6] used this methodology to carry out an *in vitro* synthesis of ageratriol (**18**) from its corresponding diepoxide (**17**) (Scheme 5.9).

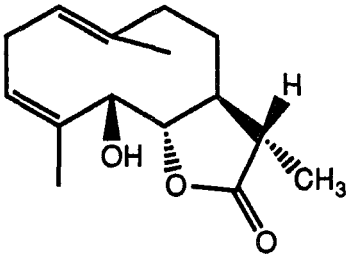
Both of the above methodologies were used to attempt a Hoffman-type reductive opening of the 4,5-epoxide of dihydroparthenolide (**14**) (DHP).

Results and Discussion

An attempt was made to carry out the reductive opening of the 4,5-epoxide of DHP (**14**) with $Al(iOPr)_3$ in refluxing toluene. After standard work-up procedures,[3] and following column chromatography, the transannular cyclization product dihydromichelliolide (**19**) was isolated (Scheme 5.10). The ^1H NMR data for compound **19** is identical to the major product resulting from the acid-catalyzed transannular cyclization of DHP (**14**) with BF_3-ether.[7] It is not known whether this cyclization occurs under the reaction conditions or whether it occurs during work-up when the residue is dissolved in a 1:1 mixture of ethyl acetate and 2N HCl. Acid is required in the work-up to liberate the allylic alcohol from the



14 15

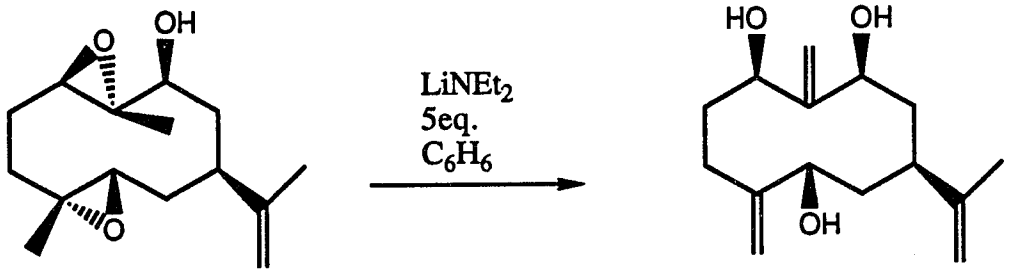


16

(i) $Al(iOPr)_3$/toluene/reflux

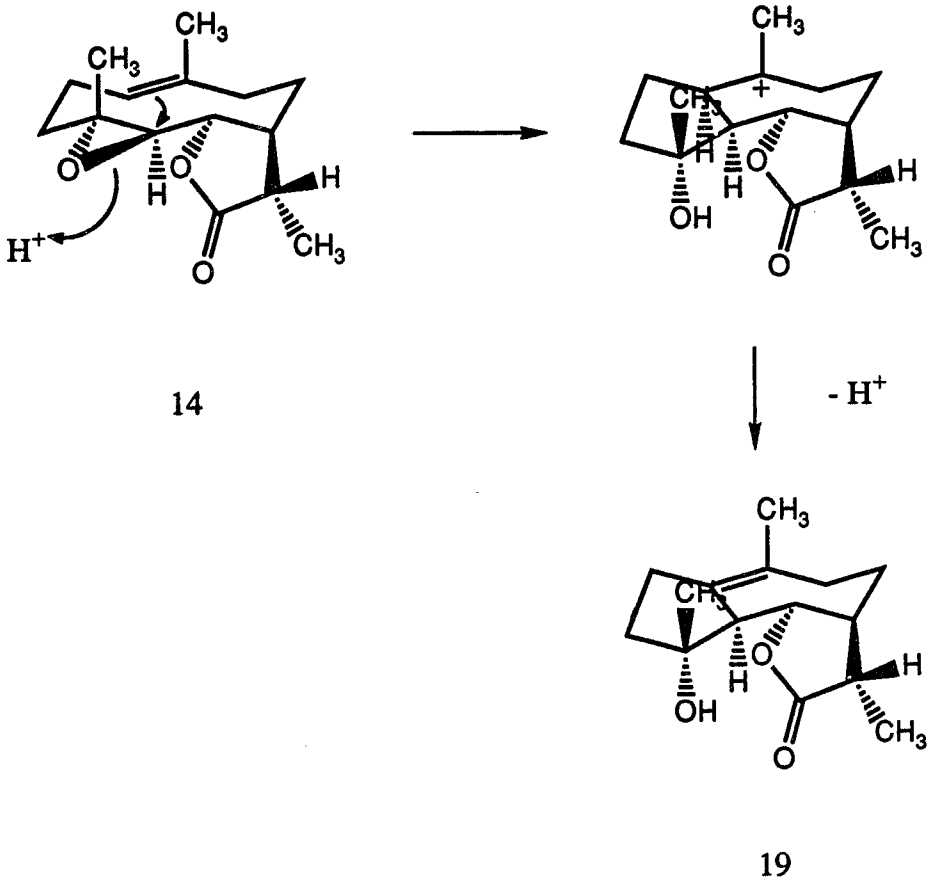
(ii) LDA/THF/reflux

Scheme 5.8



17 18

Scheme 5.9



14

19

Scheme 5.10

aluminum alkoxide intermediate[8,9] (Scheme 5.11).

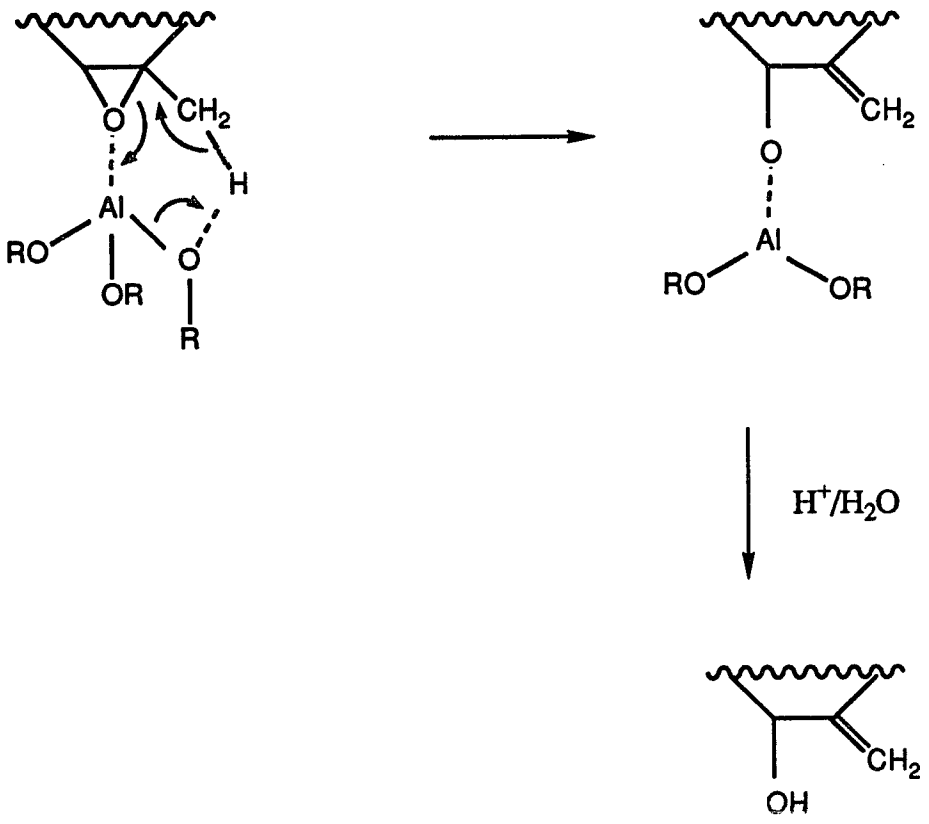
An attempt was made to carry out the same conversion with lithium diisopropylamide (LDA). After refluxing DHP (**14**) and LDA in tetrahydrofuran (THF) for 1.5hrs, the solution changed to a dark brown color. After standard work-up procedures, ^{1}H NMR analysis showed no methyl singlets in the region of 1.3-2.0ppm indicating extensive decomposition of the starting material.

Experimental Section

Reductive opening of epoxide with Al(iOPr)$_3$. A solution of dihydroparthenolide (**14**) (200mg,0.8mmol), and Al(iOPr)$_3$ (327mg,1.6mmol) in 50ml of anhydrous toluene was refluxed for 10hrs. An additional 327mg of Al(iOPr)$_3$ was added and the solution was refluxed for another 10hrs. The solvent was evaporated and the residue was taken up in a 1:1 mixture of ethyl acetate (EtOAc) and 2N HCl. The organic layer was separated and the aqueous layer was extracted with EtOAc. The organic layers were combined and washed with sat. $NaHCO_3$ and water, and then concentrated. Dry column silica gel chromatography eluting with DCM/acetone (10:1) was used to separate compound **19**.

Dihydromichelliolide (19) ^{1}H NMR (Fig. 5.7): δ 3.80 (dd,1H,C_6-H,J=10Hz), 2.60 (d,br,1H,C_5-H), 1.67 (s,3H,C_{14}-CH_3), 1.29 (s,3H,C_{15}-CH_3), 1.22 (d,3H,C_{13}-CH_3,J=7Hz).

Reductive opening of epoxide with LDA. A solution of DHP (**14**, 200mg, 0.8mmol) and LDA (1.6ml, 2.4mmol) in THF was refluxed. After 1.5hrs., the color of the solution changed from an initial orange to a dark brown. The reflux was stopped, the solution was cooled, water was added, and the solution was neutralized with 0.5N HCl. The solution was extracted with diethyl



Scheme 5.11

ether (10 x 10ml). The ether extract was dried over anhydrous Na_2SO_4, filtered, and the solvent was evaporated. TLC and ^1H NMR analysis of the residue showed signs of decomposition.

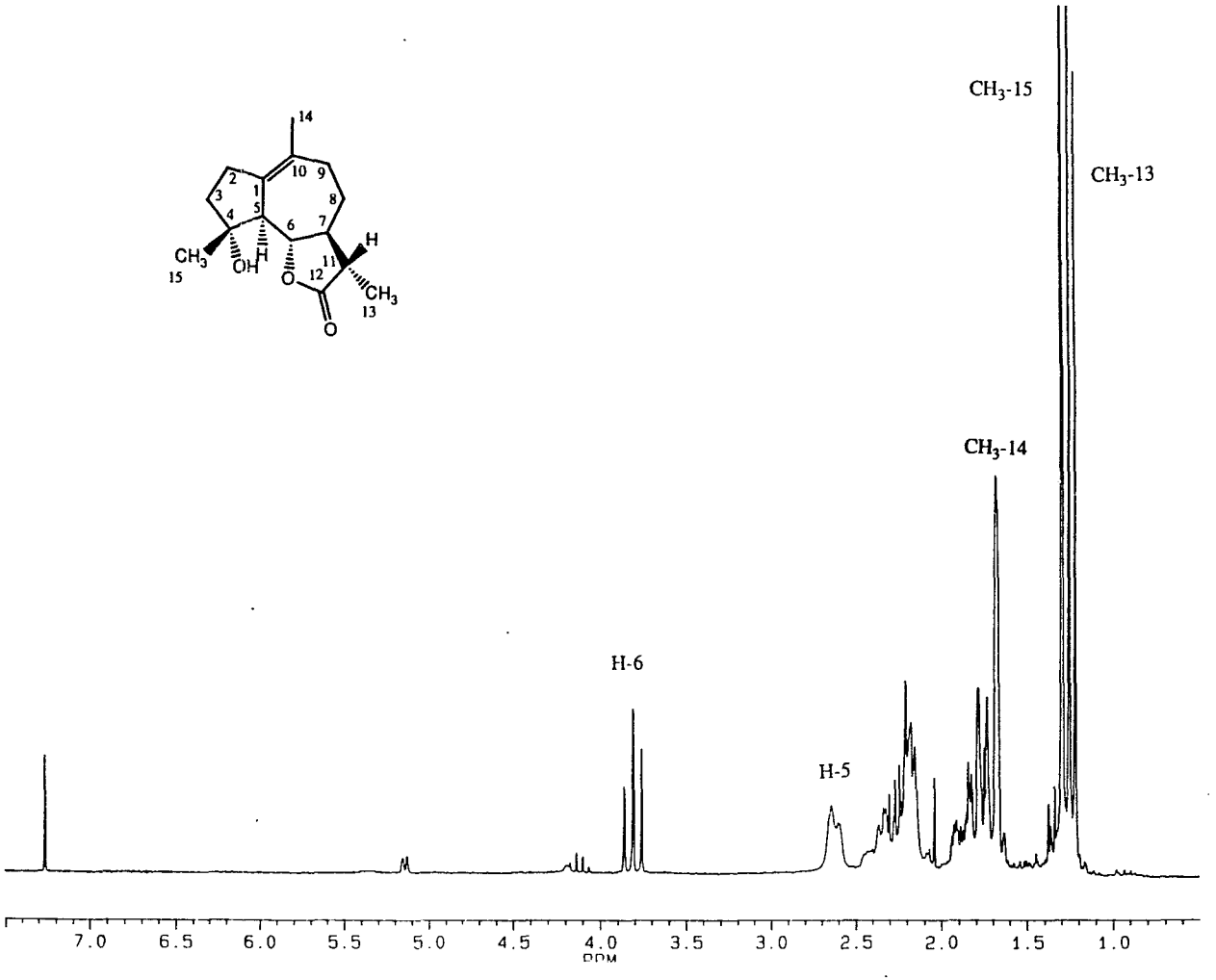


Figure 5.7. ^{1}H NMR spectrum of dihydromichelliolide (**19**) in $CDCl_3$.

References

1. Barton, D.H.R. *J. Chem. Soc.* **1953**, 1027-40.
2. Siegel, S.; Smith, G.U. *J. Amer. Chem. Soc.* **1960**, *82*, 6082-7.
3. Ando, M.; Akahane, A.; Yamaoka, H.; Kahei, T. *J. Org. Chem.* **1982**, *47*, 3909-16.
4. Lee, Ihl Young, Dissertation. "New Sesquiterpene Lactones From The Genera *Calea* And *Berlandiera* (Asteraceae) And The Fragmentation Reactions of 1,3-Dihydroeudesmanolide Derivatives." Louisiana State University, **1983**.
5. Ando, M.; Tajima, K.; Takase, K. *J. Org. Chem.*, **1983**, *48*, 1210-6.
6. Bellesia, F.; Pagnoni, U.M.; Trave, R. *Tetrahedron Lett.* **1974**, *14*, 1245-8.
7. Parodi, F., Dissertation. "Structure Elucidation of Natural Products From Asteraceae Using Modern NMR Techniques and Biomimetic Transformations of 11,13-Dihydroparthenolide." Louisiana State University, **1988**.
8. Eschinasi, E. H. *J. Org. Chem.* **1970**, *35*, 1598-1600.
9. Eschinasi, E. H. *Israel J. Chem.* **1968**, *6*, 713-21.

Part E. Hydrolysis and Relactonization of Sesquiterpene Lactones

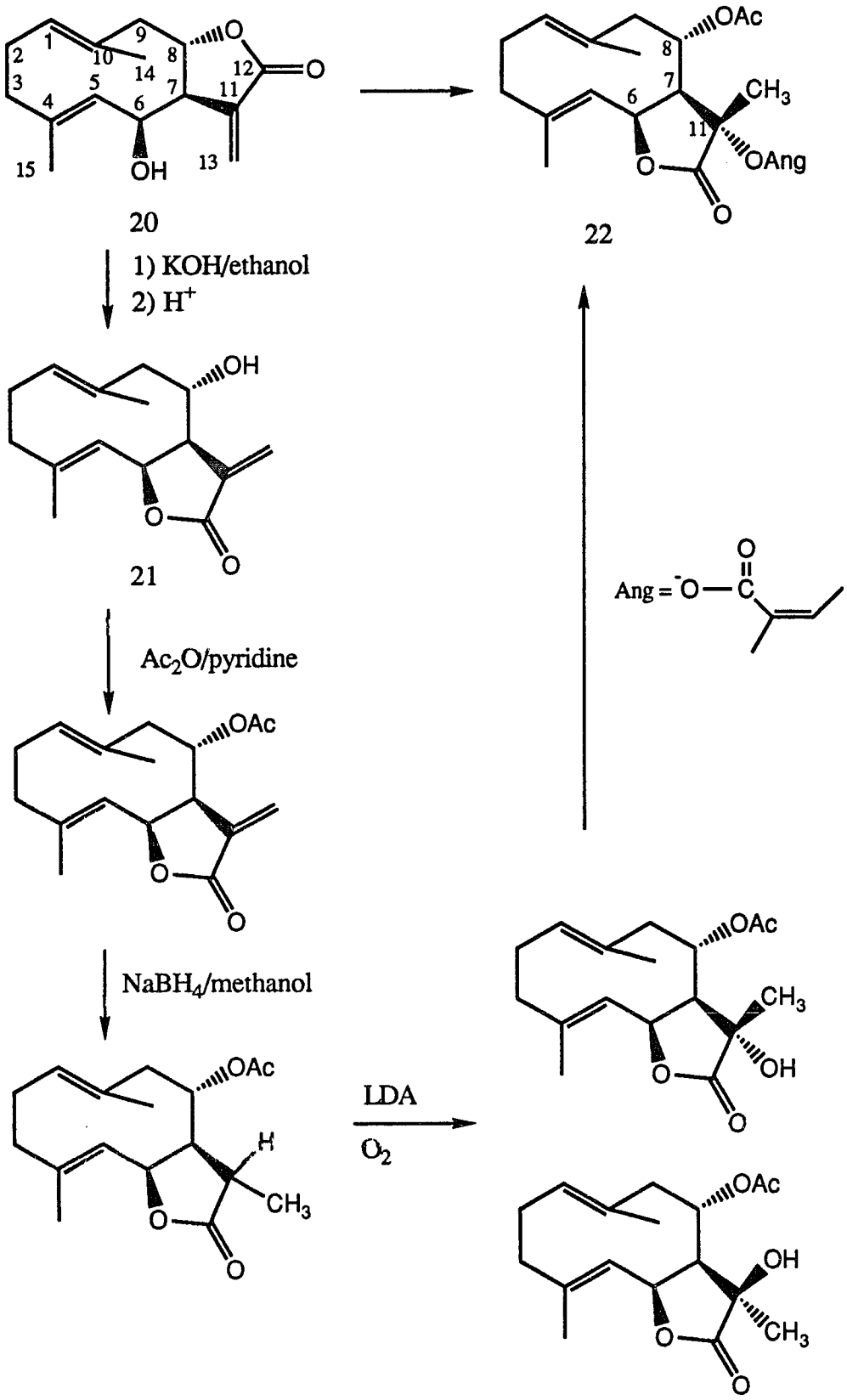
Introduction

A whole series of 11-hydroxy-6,7-cis-sesquiterpene lactones were isolated from the roots of *Laser trilobum* by Smitalova et al.[1] 11-Hydroxy-sesquiterpene lactones may be molluscicidal compounds and may be potent inhibitors of the enzyme phosphofructokinase.[2] Conversion of 6-epi-desacetyl-laurenobiolide (**20**), a naturally occurring sesquiterpene lactone isolated from *Montanoa grandiflora*[3], to laserolide (**22**) would provide a convenient route to this series of conpounds (Scheme 5.12).

Results and Discussion

Basic hydrolysis of 6-epi-desacetyl-laurenobiolide (**20**) followed by acidic relactonization could result in either C-6β,7-cis lactonization or reformation of the original C-7,8α-trans lactone (**20**). Yoshioka et al.[4] reported the hydrolysis and relactonization of C-6α,7-trans lactones to C-7,8α-trans lactones indicating the C-7,8α-lactones to be more themodynamically stable. It is not known what effect a beta-orientation of the hydroxy group at C-6 (compound **20**, Scheme 5.12) will have on the relactonization.

6-Epi-desacetyl-laurenobiolide (**20**) was hydrolyzed under aqueous basic conditions. When the aqueous solution was acidified, the C-7,8α-lactone (**20**) was isolated. Apparently, steric effects from methyl groups 14 and 15 or medium ring strain prevent lactonization to a C-6α,7-cis-lactone (**21**) and favor relactonization to 6-epi-desacetyl-laureobiolide (**20**) .


20
1) KOH/ethanol
2) H+
21
Ac2O/pyridine
NaBH4/methanol
LDA
O2
22
Ang = -O—C
OAc
OH
OAng
CH3

Scheme 5.12

Experimental Section

6-Epi-desacetyl-laurenobiolide (**20**) (70mg) was dissolved in 10ml of aqueous 10% potassium hydroxide. This solution was stirred at room temp. for 1.5hrs. (complete dissolution). The solution was acidified to a pH of 5-6 by slow addition of 0.5N hydrochloric acid. The aqueous solution was extracted with dichloromethane (DCM) (4 x 10ml). The DCM solution was dried over anhydrous Na_2SO_4, filtered, and the solvent was evaporated. The ^1H NMR data for the residue is identical to that for lactone **20** (see Chapter 4, Part D, Experimental Section).

References

1. Smitalova, Z.; Budesinsky, M.; Sama, D.; Holub, M. *Collect. Czech. Chem. Commun.* **1986**, *51*, 1323-39.
2. Pentes, H.; Fischer, N.H., unpublished results.
3. Quijano, L.; Calderon, J. S.; Gomez, G. F.; Lopez, P. J.; Rios, T.; Fronczek, F. R. *Phytochemistry*, **1984**, *23*, 1971-4.
4. Yoshioka, H.; Renold, W.; Mabry, T. J. *J. Chem. Soc., Sec. D., Chem. Comm.*, **1970**, 148-9.

Vita

Howard Gordon Pentes was born on August 6, 1963 in New Orleans, Louisiana, the son of Gerson and Sonia Pentes. He attended public schools in New Orleans, including the selective Benjamin Franklin Senior High School from which he graduated in 1981. As a senior in high school, he was concurrently enrolled at Tulane University where he successfully completed two courses.

He continued his undergraduate education at Louisiana State University in Baton Rouge, Louisiana where he obtained a B.S. degree in Chemistry in December of 1985.

He began graduate school at Louisiana State University in the spring semester of 1986. He is currently a candidate for the Ph.D. degree with a major in Organic Chemistry. His research area, working under the direction of Dr. Nikolaus H. Fischer, is in the field of Natural Products Chemistry with special emphasis on the oxidative synthesis of sesquiterpene lactones.

He is a member of The American Chemical Society and Phi Lambda Upsilon (National Honorary Chemical Society).

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Howard G. Pentes

Major Field: Chemistry (Organic)

Title of Dissertation: Oxidative Chemical Transformations of Sesquiterpene Lactones.

Approved:

Nicholas H. Fischer
Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Frank R. Cantrell

Steven F. Watkins

D. K. Carpenter

Joe P. Foley

Michael L. Hlavinka

Date of Examination:

February 18, 1991